Acta

OTO-LARYNGOLOGICA

VOL. 73 JANUARY–JUNE 1972 NO 1–6

EDITOR. C. A. HAMBERGER STOCKHOLM

ACTA OTO-LARYNGOLOGICA 73, 1–6, 1972 NARVAVÄGEN 16, 115 23 STOCKHOLM

Acta Otolaryng 73 1–3 1972

EVIDENCE FOR A TEST OF DYNAMIC OTOLITH FUNCTION CONSIDERED IN RELATION TO RESPONSES FROM A PATIENT WITH IDIOPATHIC PROGRESSIVE VESTIBULAR DEGENERATION

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(Received May 10, 1971)

Abstract. A patient is described who possessed residual otolith function, but whose loss of canal function was complete for the horizontal and nearly complete for the vertical canals. A clear (but abnormal) nystagmus response was elicited during rotation about an Earth-horizontal axis, confirming the conclusion, based on animal experiments, that this response depends upon the otolith system. This test appears to measure dynamic otolith function and therefore provides a useful supplement to other vestibular tests.

When man is rotated at a constant speed of 60 deg/sec about an Earth-vertical axis with the head positioned so that the horizontal canals are in the plane of rotation, primary vestibular nystagmus subsides about 40 sec after the initial angular acceleration ends. When the rotation axis is horizontal but all other aspects of the rotation are the same, nystagmus persists for as long as the rotation continues (Guedry 1965 Benson & Bodin 1966) The important difference in these two stimulus situations is as follows: When the rotation axis is vertical, the vestibular structures maintain a constant orientation relative to gravity and it is well known that the horizontal vestibular nystagmus in this situation depends upon stimulation of the horizontal semicircular canals by angular acceleration. In contrast, when the rotation axis is horizontal, the vestibular structures undergo continual reorientation relative to gravity and although the initial horizontal nystagmus is partially attributable to angular acceleration, the long persisting response derives from some additional sensory input which is not dependent upon angular acceleration but is dependent upon change in orientation relative to gravity

This additional input comes from the vestibular structures men with bilateral loss of labyrinthine function do not yield an unequivocal nystagmus response during rotation about an Earth-horizontal axis, and their subjective experiences are quite different from those of men with normal function (Guedry 1965). Conflicting views have been expressed concerning the specific vestibular mechanisms responsible for this kind of persistent nystagmus. Benson & Bodin (1966) postulated a novel mode of semicircular canal stimulation, whereas Guedry (1965) suggested that the response is caused by stimulation of the otolith organs. Two animal experiments support the latter hypothesis. Janeke et al. (1970) found that sectioning of the utricular nerve in rabbits abolished sustained nystagmus during rotation about an Earth-horizontal axis. Correia & Money (1970) reported that a clear nystagmus response during rotation about an Earth-horizontal axis was still present in cats after all six semicircular canals had been blocked,

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even though no response could be elicited in these operated animals by strong angular acceleration about an Earth-vertical axis. Taken together these two experiments on animals indicate that the sustained nystagmic response during rotation about an Earth-horizontal axis is provoked by the otolith system. Recent study of a clinical case appears to extend this conclusion to man A complete description of the patient appears elsewhere (Graybiel et al. 1971 in press) only the findings relevant to the present discussion are summarized herein

CASE REPORT

The subject, a healthy appearing man 26 years of age, had always been well and active except for persistent dizzy spells since childhood, which had been ascribed to "nerves" After a careful evaluation of the vestibular organs at the Naval Aerospace Medical Research Laboratory in Pensacola, a diagnosis was made of "idiopathic progressive vestibular degeneration" Hearing was normal Variability in test results and spontaneous attacks of dizziness and vertigo indicated that the pathological process was active at the time he was being tested Three years later loss of vestibular function was found to be virtually complete.

The significance of this case to the present discussion lies in the pattern of functional loss at the time the tests were conducted. Thermal stimulation and strong angular acceleration about an Earth vertical axis were used to assess semicircular canal function. These tests indicated that horizontal canal function was either severely reduced or absent. Otolith function was assessed by the ocular counterrolling (Miller 1962) and the oculogravic illusion (Graybiel & Clark 1965) tests. Scores on the former were low but normal and on the latter were well within the normal range. Thus both tests indicated the presence of otolith function.

The patient was rotated at 10 rpm about an Earth-horizontal axis for 90 sec on two separate days. On each occasion he displayed a clear

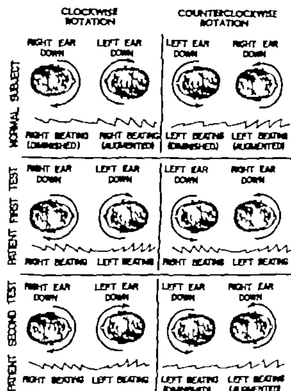


Fig 1 Comparison of patient's response with a typical normal response. Nystagmus illustrations are drawings (not actual records) to clarify the nature of the differences between the patient and a normal person. Second test was performed 10 days after first.

nystagmus response and thus differed from men without labyrinthine function who do not yield any clear nystagmus in this situation. However the patient's nystagmus response also differed from the response elicited from normal individuals, as illustrated in Fig. 1 During rotation in a clockwise direction a normal subject displays a right-beating nystagmus which is diminished as he rotates through the right-ear-down position and is augmented as he rotates through the left-ear-down position. During counterclockwise rotation he displays a left-beating nystagmus which is diminished as he rotates through the left-ear-down position and enhanced as he rotates through the right-ear-down position (Fig. 1 upper panel). On the first day of testing, the patient displayed a nystagmus which reversed direction from right-beating to left-beating just after the nose-down position was passed, irrespective

of the direction of rotation (Fig. 1 middle panel). Ten days later the patient was tested again. On this day he had experienced dizziness prior to testing, and he possessed a strong left beating spontaneous nystagmus. During clock wise rotation about an Earth horizontal axis, he exhibited the same type of direction reversing nystagmus he had displayed on the first day of testing. During counterclockwise rotation, his nystagmus response was comparable to that of a normal individual (Fig 1 lower panel). Thus the positional modulation of his response to clockwise rotation was abnormal on both days, while his nystagmus during counterclockwise rotation changed from one test to the next, indicating that the vestibular response system was in a state of change, probably due to an active pathological process.

DISCUSSION

The nature of the pathological process was such that, at the time he was tested, the patient still possessed otolith function while horizontal canal function had been lost and vertical canal function was severely reduced. Thus his vestibular system functionally resembled those of the cats whose otolith function remained intact after their semicircular canals had been surgically blocked by Correia & Money (1970) However in at least one important respect, his vestibular system probably differed from those of Correia & Money's cats. Correia & Money were able to block the semicircular canals without damaging the ampullae. Therefore, it is likely that spontaneous inflow from the horizontal canal cristae was normal. On the other hand, the pathological process seen in this patient can reasonably be presumed to have eliminated spontaneous activity The fact that the patient displayed a clear horizontal nystagmus response during rotation about an Earth-horizontal axis supports the conclusion that this response depends upon the otolith system. In addition, the indication is that the response may be independent of spontaneous inflow from the semicircular canals.

On the basis of present evidence it appears that Earth-horizontal axis rotation, which yields an easily quantified nystagmus response might provide a useful supplement to other tests of otolith function.

ZUSAMMENFASSUNG

Ein Patient wird beschrieben, der noch Reste der Otolith-Funktion besass, jedoch einen vollständigen Verlust des horizontalen und einen nahezu vollständigen des vertikalen Bogenganges hatte. Eine klare (jedoch abnorme) Nystagmus-Reaktion während der Rotation um eine erd-horizontale Achse wurde hervorgerufen und bestätigte somit die auf Tierexperimente basierte Schlussfolgerung, dass diese Reaktion vom Otolith-System abhängt. Dieser Test misst offenbar die dynamische Otolith-Funktion und liefert damit eine nützliche Bereicherung anderer vestibulärer Tests.

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Acta Otolaryng 73 4-9 1972

THE ACOUSTICO-VESTIBULAR FUNCTION DURING DUMPING PROVOCATION

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(Received April 26, 1971)

Abstract. Following gastric surgery eight patients with severe dumping syndrome and seven healthy patients were tested by way of a caloric test with electronystagmography before and during dumping provocation with glucose and apomorphine respectively Further in four of the same dumping patients and in three of the same healthy patients the acoustic function was tested under the same experimental conditions. During dumping provocation a massive, reproducible inhibition of nystagmus could be registered, whereas no changes occurred in the acoustic function, either objectively or subjectively Injection of apomorphine produces the same changes as administration of glucose, clinically experimentally and with regard to nystagmus.

One severe, incapacitating dumping syndrome (SD) is an untoward result of gastric surgery with by-pass of the pyloric sphincter The incidence of SD is only a few per cent, but the syndrome is rightly considered a dreaded sequela to gastric surgery The syndrome may be reproduced constantly by intra-intestinal administration of hypertonic glucose solutions.

It has not been possible to register changes specific for SD but patient suffering from SD are characterized by their pronounced sensitivity respectively low threshold to 1) hypertonic glucose solution in the small bowel (Meurling 1953 Borgström 1960 Fenger 1962, 1967). 2) apomorphine (Borgström, 1964 Fenger & Gudmand-Høyer 1968 Gudmand-Høyer et al., 1968) 3) carbon dioxide in the respiratory centres (stimulated ventilation) (Borgström & Bülow 1967)

Sensitivity to these agents facilitates the application of preoperative tests prior to gastric surgery (Hinshaw et al., 1960 Fenger 1962, 1967 Borgström & Bülow 1967 Fenger & Gudmand-Høyer 1968 Gudmand-Høyer et al. 1968) Apomorphine has its specific point of attack in the brain stem and its administration provokes dumping symptoms qualitatively and quantitatively identical to the symptoms elicited by intra-intestinal administration of hypertonic glucose solutions (Borgström 1964 Fenger & Gudmand-Høyer 1968 Gudmand-Høyer et al., 1968). As the cochlear and vestibular nuclei in the brain stem have numerous connections, especially to the reticular formation, investigations of the acoustico-vestibular function during dumping provocation with glucose and apomorphine have been carried out.

MATERIAL

The material comprises 8 patients with severe dumping and 7 healthy patients who had previously undergone gastric surgery In each group 6 patients had had a subtotal gastric resection performed Vagotomy and pyloro-

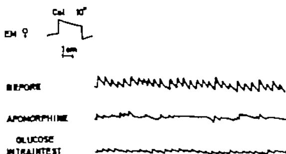


Fig. 1. Caloric induced nystagmus in a patient with severe dumping.

plasty had been carried out in 2 of the patients in the group with SD and in one of the cured patients. All patients were examined at least 1 year after gastric surgery.

METHODS

Prior to the investigation repeated provocations with glucose as well as apomorphine were carried out in all 15 patients until a constant reaction was obtained ensuring the adaptation of the organism to the dumping stimulus (Fenger 1967). Vasovagal attacks did not occur. Spontaneous dumping has been classified according to Meurling's (1953) and Fenger's (1967) definitions, experimental dumping according to Fenger's (1967).

The evaluation of nystagmus has been carried out by means of electronystagmography (ENG) (Aschan et al. 1956, Jongkees & Philipszoon 1964). Nystagmus was provoked by caloric stimulation, viz. by irrigating the external auditory meatus with water (30 C) for 40 seconds.

All 15 patients were subjected to the following series of ENG examinations. Auditory investigations were carried out in 4 of the patients with SD and in 3 of the healthy patients. In the fasting patient a control ENG with calorization is primarily carried out (Fig. 1). ENG is then repeated during simultaneous dumping provocation with subcutaneous injection of $^1/_2$ mg apomorphine. At a later date ENG is carried out during a dumping provocation with 50 g glucose dissolved in 250 ml water applied intra-intestinally. In the dumping patients nystagmus is registered while the dumping symptoms are maximal; in the healthy patients it is registered 20 min after the start of the examination, this time corresponding to the peak of the dumping symptoms in the former group.

Nystagmus is evaluated by its duration in seconds and by maximum eye speed of the slow component expressed in degrees per second. The maximum eye speed is the most important parameter (Henriksson, 1955, Pettersen, 1965) as it expresses the magnitude of nystagmus.

The acoustic function was evaluated by means of tone audiometry and stapedial reflex examination (Terkildsen, 1962) in order to establish the subjective as well as the objective threshold of hearing. Then dumping provocation with apomorphine and, on another day, with glucose was carried out and during these provocations tone audiometry and stapedial reflex examinations were again performed at

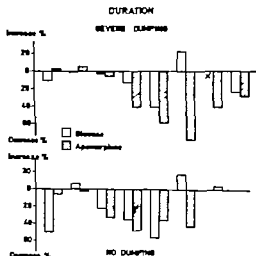


Fig. 2. The changes in duration of nystagmus expressed in percentage of the control examination during dumping provocation with glucose and apomorphine. — = not performed.

intervals of 5 min lasting 45 min from the start of the provocation.

RESULTS

During dumping attacks no spontaneous or positional nystagmus has been registered indicating a stimulation. Fig. 1 shows a massive inhibition of nystagmus during both provocations as compared with the control examination.

The duration of nystagmus expressed in percentage of the control examination is shown in Fig. 2. In most cases a pronounced inhibition is registered following glucose as well as apomorphine provocation and independent of *the presence or absence of a dumping reaction.*

Maximum eye speed is shown in Fig. 3 for the same two groups and illustrated in the same way. A pronounced inhibition is again found in most patients following glucose as well as apomorphine provocations and irrespective of the presence or absence of the dumping reaction. The massive inhibition occurring during the dumping attack ceased towards the end of the attack as shown in a investigation of 3 patients. It should stressed that the dumping reaction, as ex d by the patient, remains unchanged the calorization and that the inhibitory effect is reproducible. No significant differences in the vestibular reactions have been found when a comparison has been drawn between the healthy patients and the dumping patients, and between glucose and apomorphine provocations.

The results of the acoustic examinations are illustrated in Figs. 4 and 5 showing two audiograms for each patient. Each of these audiograms shows the changes in the threshold intensity at tone audiometry (upper rows) and stapedial reflex examination (lower rows). For each *frequency a threshold value is indicated* during the period 0–45 min registered every fifth minute. No significant changes in the threshold at any of the frequencies occurred during the 45-min period of observation.

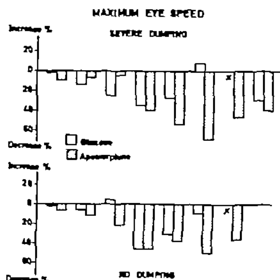
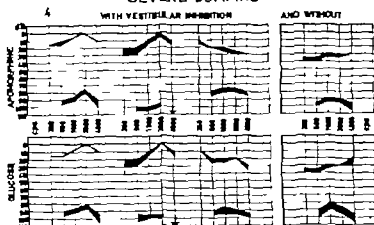


Fig. 3. The changes in maximum eye speed of nystagmus expressed in percentage of the control examination during dumping provocation with glucose and apomorphine. — = not performed.

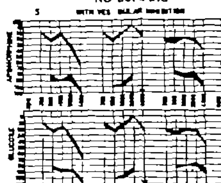
DISCUSSION

Since Machella (1949) showed that hypertonic solution in the small bowel is the primary eliciting factor in SD the cardiovascular changes and theories have played a dominant role in most studies of the pathophysiology of SD. In recent years a number of publications have expressed certain doubts concerning the validity of their dominant role (Borgström, 1960 Butz, 1961 Christoffersson, 1965 Strandness & Bell, 1967). During dumping provocation with apomorphine in 7 patients with SD our group (Fenger et al., 1971) found no significant cardiovascular changes (Fig. 6) although the administration of glucose (50 g/250 ml water) resulted in the same severe dumping symptoms as those provoked by apomorphine.

During dumping provocations in which apomorphine was used as well as during provocations with glucose we have found a massive, *unspecific inhibition of the vestibular function* but in no case was a similar inhibition of the acoustic function registered. The explanation to this must be the fact that the inhibitory impulses arising during the dumping provoca-



Figs. 4–5. Changes in threshold intensity of tone audiometry (upper rows) and stapedial reflex examination (lower rows) after dumping provocation with apomorphine and glucose observed during 45 min.



tion reach the central parts of the vestibular system but not the acoustic centres localized in the brain stem, which may again be explained by the anatomy of the brain stem (Brodal et al., 1962 Blegvad, 1970)

Auguste (1954) states, contrary to the findings of this material, that an acoustic inhibition may be registered synchronous with the dumping symptoms. The variations in hearing are small, however and involve only a few frequencies, and it may well be a question of the patient's drowsiness as the acoustic examination was purely subjective and did not, as in this material, involve an objective evaluation by means of a stapedial reflex examination. During his vestibular investigations, Auguste used galvanic stimulation, and in 5 patients with dumping he found a lower threshold for the vestibulo-spinal reaction. This may be explained by the patients hypotony

Apparently Auguste has not registered any changes in the caloric-induced nystagmus. It should be stressed that the very pronounced inhibition of nystagmus is not caused by vasovagal attacks as these did not occur because of the repeated provocations until constant reaction, which had been carried out prior to the investigations (Fig. 6)

The *considerable inhibition of nystagmus* during dumping provocations has not been described earlier It is very interesting that the intra-intestinal administration of glucose should lead to the same massive inhibition as *that released by the apomorphine.* The massive inhibition released by glucose is assumed to stem from the small bowel, and may be an expression of an unspecific irradiation of strongly inhibitory impulses from the small bowel to the vestibular system. However the investigations do not indicate whether these impulses reach the vestibular system from higher or lower levels of the central nervous system, nor do they prove that the inhibition is responsible for the dumping experience of the patient.

The fact that injection of apomorphine

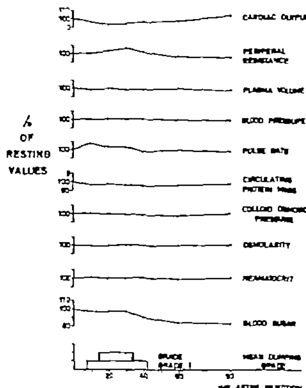


Fig. 6 Mean changes in percentage of resting values of cardiovascular parameters during apomorphine provoked severe dumping in 7 patients after subtotal gastrectomy. Dumping grade I (□): the patient feels distinctly dizzy, drowsy, sleepy or tired. Grade II (□): the patient feels a distinct, although resistable, desire to lie down.

duces the same changes as administration of glucose supports the clinical observation that the dumping reaction provoked by apomorphine is qualitatively as well as quantitatively the same as the dumping reaction provoked by glucose.

ZUSAMMENFASSUNG

Acht Patienten mit schwerer Dumping-Syndrom und sieben in Bezug auf diese Sache von Natur aus raschen Patienten wurden nach Magenchirurgie mit Elektronystagmografie registrierter calorischer Prüfung vor und während der Dumpingprovokation mit teils Glukose und teils Apomorphin untersucht. Danach wurden vier von den Dumpingpatienten und drei von den von Natur aus raschen Patienten unter denselben experimentellen Bedingungen mit Hörproben untersucht. Es wurde während der Dumpingprovokation eine massive, reproduzierbare Hemmung des Nystagmus gefunden. Veränderungen des Hörvermögens hat man dabei aber nicht registrieren können, weder objektiv noch subjektiv. Aus parenteraler Einbringung von Apomorphin erfolgen dieselben klinischen und experimentellen Veränderungen und dieselben Veränderungen des Nystagmusmusters wie aus intraintestinaler Einbringung von Glukose.

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Acta Otolaryng 73 10–17 1972

MORPHOLOGY OF THE STAPEDIOVESTIBULAR JOINT[1]

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(Received May 10, 1971)

Abstract A series of temporal bones taken from humans and laboratory animals was examined by light and scanning electron microscopy for the incidence, size and location of articular spaces in the stapediovestibular joint. Joint cavities were found in 70% of adult human joints. None were found in children. The majority were located in the posterior pole of the joint. On the average, they extended through one-tenth of the length of the circumferential joint. Comparative anatomy of the joint in laboratory animals was found to differ significantly among various species.

A knowledge of the anatomy of the union between the stapes and the oval window is essential to understanding the pathologic mechanisms of stapedial ankylosis and the motions of the stapes in the oval window While studies of the pathological processes affecting the joint have been advancing recently such as the scanning electron microscopic studies of otosclerotic stapes by Boenninghaus & Becker (1968) Clarke (1969) and Lim (1970) knowledge of the precise anatomy of the joint has not kept pace. The ontogeny and phylogeny of the joint likewise lack documentation, and such information could aid greatly in understanding the anatomy of the joint in the adult human

The structure of this joint in humans has been the subject of some controversy Most anatomists depict it as consisting entirely of a fibrous annular ligament (Bast & Anson, 1949 Anson & Donaldson 1967) while others have described articular cavities (Toynbee, 1853 Rüdinger 1873) Engström (1948 Engström & Röckert 1962) on the other hand, stated that the joint is purely fibrous in some human specimens, but in others contains a true joint cavity He called this a "half joint."

The purpose of this study is to help establish the fine morphology of the human stapediovestibular joint and its comparative anatomy in laboratory animals.

METHODS

One hundred slides of human temporal bones were examined by light microscopy Specimens with trauma or disease directly affecting the oval window area were excluded. The age range was from stillborns to 93 years, with an average age of 44 In addition eight sets of human fetal temporal bones were examined All specimens had been cut 20 μ thick and every tenth section stained. Wherever necessary additional sections were stained. Some selected sections were stained with Alcian blue-PAS for mucopolysaccharides. Cavities were considered to be present in the stapediovestibular joint only if they persisted in more than one section and did not appear to be artifacts. The number of sections through which the joint cavity extended was

This work was supported in part by research grants from the Research Council of the American Otological Society the Deafness Research Foundation, and the NIH NINDS (5-NB5816-06)

Read before the Research Meeting of the American Academy of Ophthalmology and Otolaryngology Las Vegas, Nevada, Sept. 18, 1971



Fig 1 Posterior poles of human stapediovestibular joints showing (*a*) a clearly demarcated articular cavity ×99 and (*b*) a cavity resembling a system of lacunae (*arrow*), ×104. *S* = stapes.

recorded and used to calculate the actual size of each cavity The location of the cavity within the circumferential joint was noted. One footplate and joint containing a cavity of average length was reconstructed.

Animal temporal bones examined included 18 guinea pig, 32 cat, 38 squirrel monkey 6 chinchilla, 2 dog, and 4 sheep bones.

Five human stapes obtained from autopsies and ten animal stapes were prepared for scanning microscopy as described by Lim (1970), and the margins of the footplates examined.

FINDINGS

Of the 100 human temporal bones studied, 54 had spaces in the stapediovestibular joint. Some of the joint spaces were clearly defined cavities, often with a lining of flat cells (Fig. 1 *a*), while others consisted of a system of lacunae formed by plexiform intercommunications of the elastic fibers (Fig. 1 *b*). Almost all of the spaces were located in the posterior part of the joint adjacent to the junction of the posterior crus and the footplate (Table I) The average length of these posterior spaces

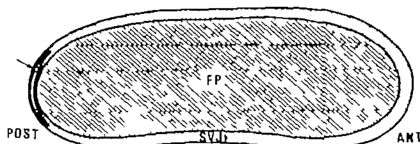


Fig 2 Reconstruction of a typical adult human foot plate (*FP*) and stapediovestibular joint (*SVJ*) with an articular cavity (*arrow*) at the posterior pole.



Fig 3 Anterior poles of human stapediovestibular joints with (*a*) radially directed elastic fibers, ×108 and (*b*) a rare finding of a joint space (*arrow*), 97 *S* = stapes.

was 0 72 mm. They extend through roughly one tenth of the total length of the circumferential joint (Fig. 2). Most anterior poles of joints consisted of radially directed elastic rs (Fig 3 *a*) but a few specimens contained apparent joint cavity (Fig. 3 *b*)

The youngest specimen exhibiting a joint cavity was 13 years old. All 22 bones in this series less than 13 years of age had purely fibrous joints, as did the eight fetal specimens. Beyond 13 years, however advancing age was not accompanied by an increasing incidence of cavities in the joints. Of the specimens over 13 years of age, 70% had cavities in their stapediovestibular joints.

Among laboratory animals studied, two types of joint were observed. The cat, dog, and sheep have a tight fibrous union with the fibers aligned in a radial pattern (Fig. 4 *a* *b* *c*) On the other hand, the guinea pig and chinchilla have a typical diarthrodial synovial joint (Fig 4 *d* *f*). The squirrel monkey joint differs from both of the above Condensations of elastic fibers bridge the joint at its tympanic and vestibular surfaces, the so-called *ligamenta baseos stapedios tympanicum* and *vestibulare* (Harty 1953) while directly between the foot plate and the oval window the joint fibers run parallel to the cartilaginous surfaces in a vestibulo-tympanic direction. Midway between the surfaces, there is a clearly demarcated cleft which extends throughout the circumferential joint. This cleft appears to be lined with flat cells.

Examination of human, monkey and guinea pig stapediovestibular joints, as well as the human incudostapedial joint, stained with Alcian blue PAS showed no apparent heavy accumulation of mucopolysaccharides in the lining cells of any of the joint spaces.

The surface topography of the footplate margins of several disarticulated stapes was examined with the scanning electron microscope. At the anterior margin in humans, there was evidence of the torn radial fibers of the ligamentous part of the joint (Fig. 5 *a*) The appearance is similar to the same location on a cat stapes (Fig 5 *b*) The posterior pole of one

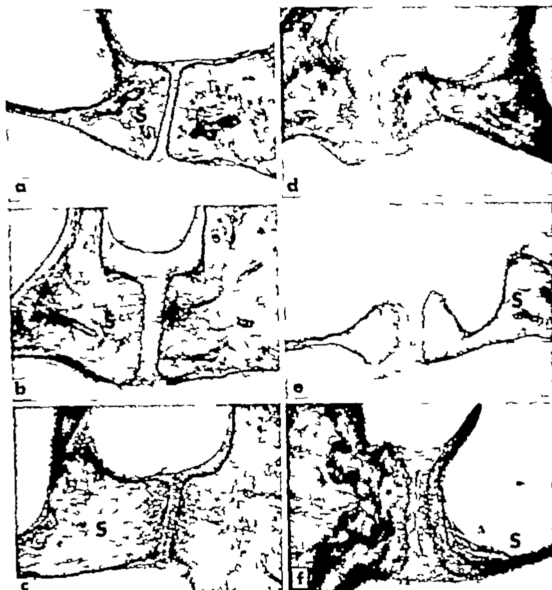


Fig 4 Stapediovestibular joints of common laboratory animals. (*a*) cat, × 88 (b) dog, 80· (c) sheep, 100, have a tight fibrous union. (*d*) guinea pig 130· (*e*) chinchilla, 79· and (*f*) squirrel monkey × 107 have a diarthrosis. *S* – stapes.

of the human footplates examined shows a smooth surface (Fig. 5 c) similar to the articular surface of a guinea pig footplate (Fig 5 *d*).

DISCUSSION

The stapediovestibular joints of common laboratory animals can be categorized into two anatomical groups: joints with articular spaces (diarthroses), and those consisting of a fibrous ligament (syndesmoses) Adding Engström's (1948) observations to our own, we find the first group includes guinea pigs, chinchillas, rhesus and squirrel monkeys. The second group includes mice, rabbits, cats, dogs, and sheep It was surprising to find animals in the same order (Rodentia) with such dir[illegible]

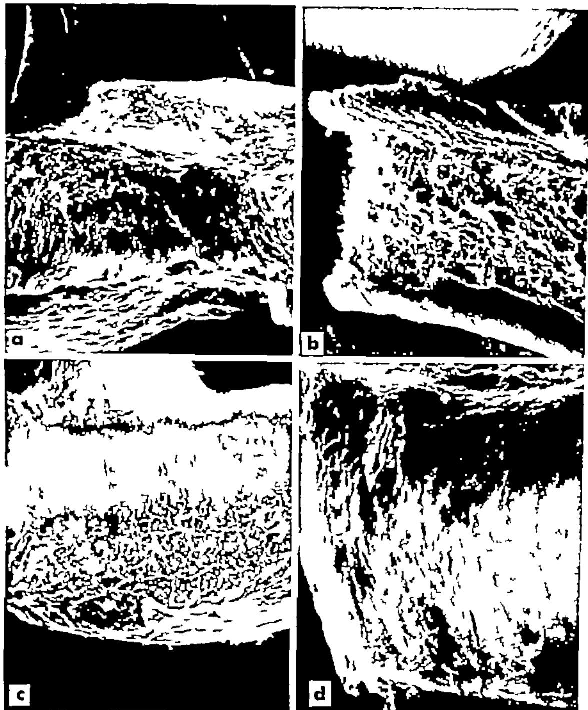


Fig 5 Scanning electron microscope views of margins of footplates of avulsed stapes. (*a*) Anterior margin of human footplate, 100, showing torn radial fibers resembling those of cat stapes (*b*), 294. (*c*) Posterior margin of a human footplate, 100 showing smooth surface of articular cavity resembling that of guinea pig stapes (*d*), 554

as the mouse and rabbit (syndesmoses) compared with the guinea pig and chinchilla (diarthroses) The monkey joints most closely resemble the human, except that joint cavities are present in every case and extend all th way around the footplate

The human stapediovestibular joint does no lend itself to such easy categorization as do th

animal joints because of variations within the species and a frequent lack of radial symmetry. Descriptions of this joint as seen in routine temporal bone sections have varied. Davies (1948) and Anson & Donaldson (1967) called it a syndesmosis, Rüdinger (1873) an amphiarthrosis, and Engström (1948, 1962) a "half joint". The present study has shown that several different configurations occur in humans. Thirty percent of adult specimens exhibited a fibrous union through the entire circumferential joint—an annular ligament. Seventy percent, however, contained an articular cleft in the posterior pole of the joint. These could be called incomplete diarthroses. Some of the clefts were clearly demarcated cavities such as Engström (1948, 1962) described (Fig. 1 *a*), while others resembled the system of lacunae seen by Rüdinger (Fig. 1 *b*).

Brunner (1873) and Gussen (1969) noted these joint spaces but interpreted them as artifacts. This possibility was carefully considered in the present study, knowing that human material is subject to post mortem autolysis and rigor mortis during the unavoidable delays before fixation. The total lack of occurrence of joint spaces in pre-pubertal specimens and the presence of flat cell linings make fixation artifact unlikely, as well as artifact caused by contraction of the stapedius muscle in rigor mortis. The discovery of similar articular clefts in other primates also suggests a natural origin. The appearance and location of the articular surface seen by scanning electron microscopy on the margin of the human footplate help verify the light microscopic findings. This articular surface (Fig. 5 *c*) resembles the articular surface in the guinea pig joint (Fig. 5 *d*), which is a diarthrosis.

The absence of cavities in the joints of children and fetuses in this series raises the questions of how and when these spaces develop. The concept of this fibrous joint cavitating into an apparent true diarthrosis well after birth becomes plausible when we consider that clefts in connective tissues resembling joint spaces are known to develop elsewhere in the body during adult life. Synovial ganglia, adventitious bursae and pseudarthroses are examples. A number of analogies can be drawn between adventitious bursae and the joint space in question in respect to their development and morphology. Both can be either unilocular or multilocular (Kuhns, 1943). Adventitious bursae develop in response to repeated trauma or prolonged friction and pressure (Crenshaw, 1963) and the same may be true for the cavities in the stapediovestibular joint. The posterior pole of the joint is the site subject to the most friction and pressure during motion of the stapes, according to Brunner (1954). He found that the ossicle has a rocking motion, pivoting on the posterior margin of the footplate when subjected to the pull of the stapedius muscle. The anterior pole of the joint undergoes more of a stretching stress, and its radially directed elastic fibers serve to help return the stapes to its resting position when the muscle relaxes.

The process by which adventitious bursae develop in connective tissues has been described by Kuhns (1943). Thickening of the loose areolar connective tissue occurs, followed by alignment of fibers parallel to the surface subjected to pressure, development of a tissue cleft containing fluid, and finally differentiation of the cells lining the cleft into a flattened, wall-like surface. The same sequence of events may occur in the stapediovestibular joint, as Fig. 6 demonstrates.

The stapediovestibular joints with joint cavities in our series may or may not be correctly called synovial joints. The development of synovial membrane cells where none existed before is a well-recognized manifestation of the ability of connective tissue to differentiate throughout life (Collins, 1949). The linings of some adventitious bursae have been noted to resemble true synovial membranes. Although opinions are divided, there is more evidence for the concept that the synovial linings of both joints and bursae are only special arrangements and modifications of connective

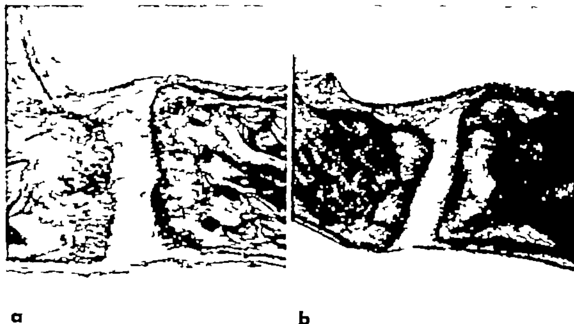


Fig 6 Possible sequence of events in cavitation of human stapediovestibular joint. (*a*) Fibers of the posterior pole of the joint in random arrangement, × 106. (*b*) Fibers aligned parallel to the surfaces bearing the pressure, × 113 *S* = stapes. Fig. 1 *a* and *b* show cavitation and flat cell lining.

tissue cells, and not true endothelial membranes (Kuhns, 1943).

CONCLUSION

Most human stapediovestibular joints are incomplete diarthroses, their posterior poles containing an articular cavity which develops after childhood, possibly in a manner analogous to the formation of adventitious bursae. The common term "annular ligament" is therefore accurate only in children, a minority of adults, and in certain laboratory animals.

ACKNOWLEDGMENTS

The authors wish to thank Dr William H. Saunders for his critical review of the manuscript, Joan Lanning, Deborah Bauman, Lawrence Irwin and Mariann Migliore for their invaluable technical assistance, and Ester Dipasupil for her assistance in preparing the manuscript.

ZUSAMMENFASSUNG

Eine Anzahl Schläfenknochen von Menschen und von Versuchstieren wurden mit dem Lichtmikroskop und mit dem Rasterelektronenmikroskop auf die Häufigkeit, Grösse, und Lage von Gelenkspalten im Steigbügelvorhofgelenk untersucht. Siebzig Prozent der Gelenke von Erwachsenen wiesen Gelenkspalten auf. Kinderknochen zeigten keine Gelenkspalten. Die meisten Spalten lagen im Crus curvilineum und erstreckten sich im Durchschnitt auf ein Zehntel der Länge des Gelenks. Ein Vergleich der Anatomie der Versuchstiere wies erhebliche Unterschiede zwischen den verschiedenen Arten auf.

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Acta Otolaryng 73 18-26 1971

HISTOLOGY OF TYMPANOSCLEROSIS

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(Received May 25 1971)

Abstract Light and electronmicroscopic studies of tissue from pathological middle ears revealed in sixteen cases changes typical of tympanosclerosis, i e. collagenous connective tissue poor in cells, with hyaline degeneration and occasional calcium deposits. In one case new formed cartilaginous tissue was present. In several specimens we found granulation tissue, which is assumed to represent the incipient stage in the pathogenesis of tympanosclerosis. Electronmicroscopy has revealed that the degenerative process involves not only connective tissue, but also the epidermis of the tympanum. It is stressed that the diagnosis of tympanosclerosis can be established only by means of microscopic study of the removed tissue.

...e histological changes in the middle ear ...t today are known as tympanosclerosis ...ave been observed for almost a century In 1873 von Tröltsch described a pathological process with considerable stiffness of the mucous membrane eventually leading to complete rigidity and calcareous or osseous degeneration. Similar changes were reported by Walb (1878) who in the middle ear found new formed connective tissue with hyaline degeneration and a scarcity of nuclei. These findings were confirmed by Pomeroy (1883) St. John Roosa (1891) and Politzer (1894) In advanced cases the new connective tissue had formed a thick, poorly vascularized fibrous coating, which gradually would fill up the entire middle ear The outcome was a fixation of the ossicles of the middle ear and obliteration of the tympanic cavity

After the turn of the century few workers showed any interest in this pathological picture, until Zöllner & Beck in 1955 published nine cases, which had undergone operation. Their paper introduced the term of tympanosclerosis.

The subsequent increase in the number of tympanoplastic operations revived the interest in the surgical aspects. The occurrence of tympanosclerosis in a large material has been calculated as 5% of all operations (Harris, 1961)

Also the number of histological studies has increased. These studies have confirmed that the predominant feature in the histological picture is a submucous formation of collagenous connective tissue, poor in cells, followed by hyaline degeneration (Brockmann, 1961 House & Sheehy 1960 Joseph & Gordon, 1963 Igarashi et al. 1970)

Several writers described calcareous deposits in the hyaline tissue (House & Sheehy 1960 Zöllner 1963) Schuknecht et al. (1966) however reported having observed this finding only very rarely

Ossification is another feature in tympanosclerosis (Sheehy & House, 1962 Zöllner 1963) Harris (1961) suggested classification in sclerosing mucositis and osteoplastic mucoperiostitis, representing different phases in the development of the pathological changes.

Zöllner (1969) and Chang (1969) carried out electronmicroscopical studies of non-decalcified tympanosclerotic material, described degenerative changes in the lamina propria,

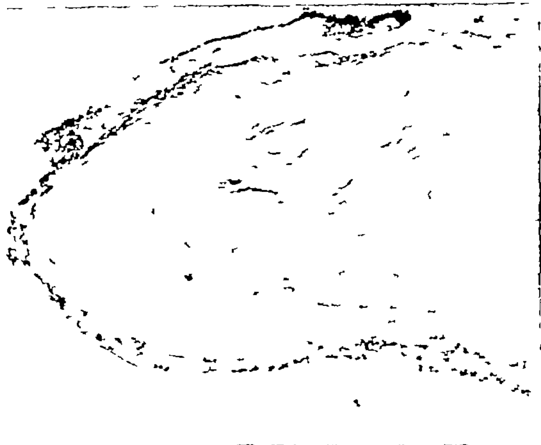


Fig. 1. Plaque in the tympanic membrane. The amorphous connective tissue shows hyaline degeneration and calcareous deposits.

and made a detailed analysis of the calcareous deposits.

Despite the large number of publications, the etiology and pathogenesis of tympanosclerosis has remained obscure. The present study is a contribution to the histology and pathogenesis of tympanosclerosis by means of light and electron microscopy.

MATERIAL AND METHOD

The material of the histological study was removed on operation from 16 patients, 9 male, 7 female, aged 15 to 66, mean age 46. The preoperative diagnosis was chronic otitis media or sequelae of middle ear inflammation.

In most cases the macroscopic changes were so pronounced, and typical of tympanosclerosis that the diagnosis was obvious even without microscopy. In these cases the middle ear was more or less filled out by thick layers of pale rubbery tissue, which also covered and sheathed the ossicles—or remnants of these, resulting in various degrees of fixation.

The tissue could be loosened in sheets and removed, layer by layer, down to the underlying osseous tissue. In other cases the typical macroscopic alterations were absent, and the diagnosis, sometimes quite unexpectedly, was made on the histologic examination. Gross examination in these cases revealed a thickened mucous membrane and recent formation of granulation-like tissue in the middle ear.

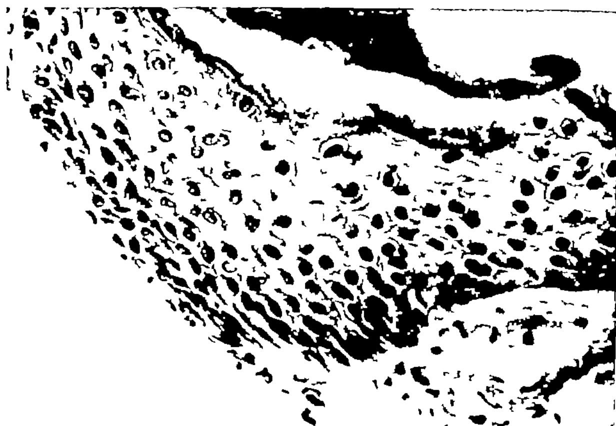


Fig 2 Cartilaginous tissue developed from connective tissue in the middle ear

l material was fixed immediately after emoval. For light microscopy specimens were fixed in cold formalin, embedded in paraffin and stained with hematoxylin-eosin Decalcification was not necessary

Most specimens were also studied by means of a Philips electron microscope by 60 kV The material was cut into smaller pieces and fixed for 2 hours at 4 C in 2.5% glutaraldehyde buffered with sodium cacodylate The specimens were washed in a 0.2 M sucrose solution in cacodylate buffer and decalcified with EDTA adjusted to pH 7 2. In some cases the specimen was not decalcified. After rinsing in sucrose cacodylate buffer and post fixation in OsO_4 the specimens were dehydrated in a series of graded ethanol and embedded in Epon. Sections of 1 μm in thickness and stained with methylene blue were used for orientation Thin sections were cut by a LBK ultramicrotome, stained with lead citrate and studied under the electron microscope

RESULTS

Light-microscopy

In all specimens there are bundles of collagenous connective tissue, poor in cells, lying in a thickened, but normally structured submucosa. In some specimens the overlying epithelium is intact.

In all of the specimens there are also typical amorphous hyaline masses, which stain metachromatically with toluidine blue. The hyaline in the periphery is markedly stratified. The vascularization is sparse. In the hyaline there are occasional elongated, dark cells.

One specimen removed from the promontorium contains connective tissue very rich in cells, with numerous elongated, dark nuclei in



Fig 3 Basal portion of the epidermis, with incipient degeneration *T* tono-fibrils. *D* desmosomes. *M* mitochondria in degeneration, *N* nucleus. *Nc* nucleolus *C* cell-borders. 27 300.

a sparse stroma, as well as tissue with a smaller number of cells, abundant intercel lular eosinophilic substance and large, light oval nuclei situated in lacunar rarefactions, i.e typical cartilaginous tissue. Between the two tissue patterns there is a gradual transition with more variegated cell forms. Around the tympanosclerotic plaque connective tissue rich in cells and vessels and roundcell infiltration is frequently seen. Calcium deposits are a fre quent finding in the hyaline plaque and in the sparse-celled collagenous connective tissue.

Electron microscopy

The study of the tympanosclerotic plaques in the tympanic membrane has been directed at 1) the basal portions of the multi-layered pavement epithelium, 2) the lamina propria, and 3) the sclerotic plaque

1) *Pavement epithelium* In the cytoplasm, cell demarcations are blurred and there is densification of the desmosomes and the tonofibrils situated on these. At the same time a blurring of the structure of tonofibrils occurs, and numerous delicate fibrils are formed throughout the cytoplasm, where numerous free ribosomes are seen. The mitochondrias lose their cristae and their elongated shape they be come more roundish, their content becomes uniformly granular whereafter they are dis solved. Numerous vesicles surrounded by a ring of ribosomes are formed. The cell nuclei become necrotic with ondensation o

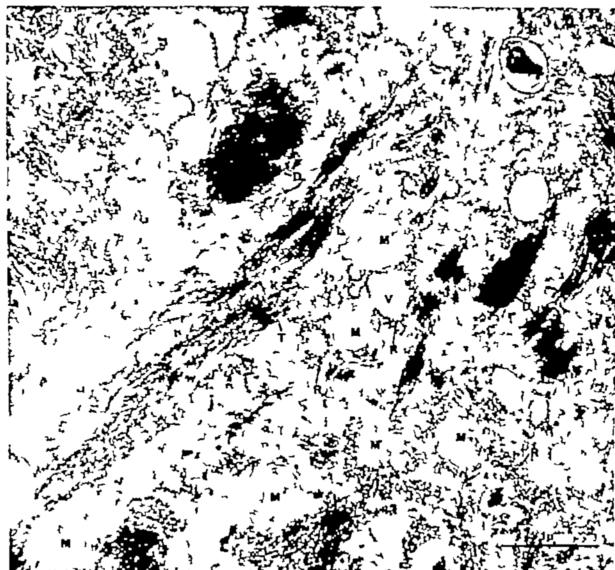


Fig 4 Basal portion of the epidermis showing various stages in the degeneration of mitochondria and desmosomes. *C* cell-borders. *D* desmosomes. *M* mitochondria. *R* ribosomes. *T* tono-fibrils. *V* vesicles. × 39 500.

chromatin and blurring of the cellular out lines.

2) *Lamina propria.* Cell nuclei and cytoplasm undergo similar changes. It is remarkable that the extremely well-developed Golgi net is preserved despite pronounced degeneration of all other cellular organelles. Lysosomal structures are also seen in the cytoplasm.

With increasing degeneration of the cells, increased amounts of delicate, cross-striated fibrils appear in the intercellular substance. Around these new formed fibrils there are numerous fine granula, which are often arranged in circular shapes.

3) *Examination of the sclerotic plaque* This reveals an amorphous basal substance containing numerous delicate fibrils with suggested cross-striation. Moreover there are dark granula, occasionally fusing into larger ring-shaped plaques with a central accumulation of granula.

A few specimens consist almost exclusively



Fig. 5. Lamina propria, advanced degeneration with remnants of fibroblasts, new formed fibrils and granulous appearance. *F* fibroblast. *Fr* fibrils. *G* granula. × 13 000.

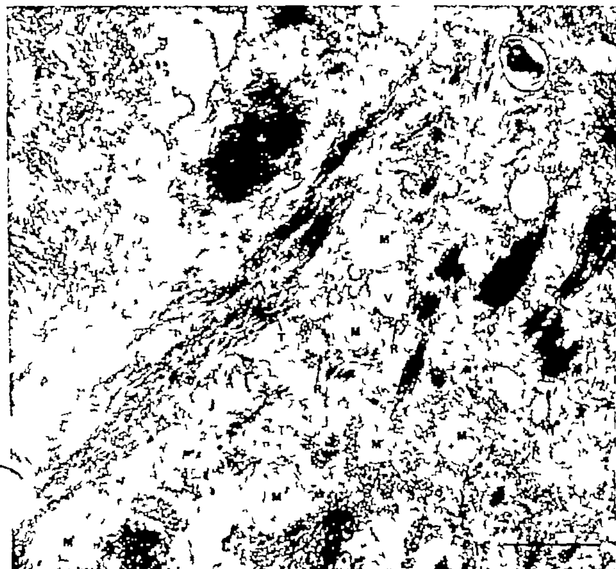


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acute and chronic inflammation, which may lead to degeneration, exudation and prolifera tion. There are two cell types. 1) mesodermal cells from outside with multipotent properties 2) cells already present in the tissue. The new formed tissue is granulation tissue In the chronic stage fibroblasts are the only cells present, and the tissue reaction is exclu sively reparative.

The first stage in the pathology of tym panosclerosis is presumably the granulation tissue during the formation of which the middle ear is invaded by mesodermal cells with multipotent properties. In the course of tissue degeneration hyaline tissue is formed and calcification may occur The mesodermal cells may be transformed into cartilaginous or osseous cells.

Even less serious lesions, as for example serous otitis, may lead to formation of connective tissue, which again may undergo hya line degeneration.

If reinfection occurs, new granulation tissue may develop and the existing tissue may undergo further alterations. It is therefore understandable that the structures of the tym panic cavity with acute and chronic inflammation may undergo severe changes, includ ing formation of tissue types like those observed in the present study

Electronmicroscopic studies have shown that the degenerative changes involve not only the lamina propria and the submucosal stratum—as so far assumed—but also the basal portions of the multilayered pavement epithelium of the tympanum.

Chang (1969) demonstrated the granulous, calcareous structure of the sclerotic plaque in non-decalcified specimens. In our material this structure appeared in decalcified and non-de calcified tissue, which stresses the organic nature of the granula.

Tympanosclerosis may be used as the com mon designation for these post inflammatory findings, which indeed are irreversible but not immutable. Finally it must be stressed that tympanosclerosis is a histological diagnosis. In the present material the typical histological changes were observed by microscopy on tissue from the middle ear that was not suspected of tympanosclerosis on gross examination.

ACKNOWLEDGMENT

Some of the material studied in this paper was received from Henrik Johansen, M.D Rigshospitalet, Tagensvej, Copenhagen.

ZUSAMMENFASSUNG

Licht und elektronenmikroskopische Untersuchungen von Gewebe aus pathologischen Mittelohren wiesen in 16 Fällen Veränderungen auf, die für Tympanosklerose typisch sind, d.h. zellenarmes, kollagenhaltiges Bindegewebe mit hyaliner Degeneration und zuweilen Kalkablagerungen. In einem einzelnen Fall wurde Knorpelgewebe vorgefunden. In mehreren Präparaten wurde Granulationsgewebe gefunden, von dem anzunehmen ist, dass es das Vorstadium zur Tympanosklerose ist. Elektronenmikroskopie hat gezeigt, dass die durch Degeneration bedingten Veränderungen nicht nur im Bindegewebe vorkommen, sondern auch — entsprechend zur Epidermis — in der Membrana Tympani. Zu betonen ist, dass die Diagnose auf Tympanosklerose nur durch Mikroskopie des entfernten Gewebes mit Sicherheit zu stellen ist.

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Acta Otolaryng 73 27–37 1972

THE LASER AS A TOOL IN INNER EAR SURGERY

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(Received June 30, 1971)

Abstract. Minute and reproducible inner-ear lesions were produced in surviving guinea pigs by using an argon laser Preselected areas, mainly in the cochlea, were irradiated for 0.5–30 seconds at power densities at the focal spot of around 1 to 2 kW/cm² The laser beam did not perforate the otic capsule but produced superficial changes. Beneath the impact point in the bone, a rounded lesion was observed in the vascular stria. Damage to the organ of Corti of varying extent occurred, depending on the energy dosage delivered. The organ of Corti in the impact area was characterized by either a complete loss or a reduced number of lipid granules in the Hensen cells. Hair-cell changes covering about one-third of a cell were frequently noted. Variations in the appearance of the cilia after irradiation are presented. Expansions of the tips of the cilia on the outer hair cells are described. The outer hair cells proved to be more affected by the laser irradiation than the inner hair cells Scanning electron microscopy and phase-contrast microscopy were used to investigate the results.

Serial experiments in which attempts were made to influence the intralabyrinthine structures of surviving animals by irradiation with laser beams have been reported previously (Stahle & Högberg, 1965 Högberg et al., 1967 Högberg et al., 1967 Högberg & Stahle, 1971). It was possible to transmit energy through the otic capsule without perforation but in sufficient quantity to destroy the sensory epithelium (Högberg et al. 1970 Engström et al. 1970) The extent of the damage was limited by a focusing device which produced a minimum diameter of the laser beam of 25 μm at the focal spot. Accurate aiming of the beam was achieved by incorporating viewing optics with the focusing lens of the laser to form a microscope with a magnification of 40×

This work was supported by the Swedish Medical Research Council (Project No. B69-17X-617-04).

Among the different models tested, the continuously operating laser has proved to be the most suitable for biomedical research at the present time because the energy dosage can be controlled accurately Two different models with such properties have been used in our experiments, a repetitive ruby pulse laser and more recently an argon laser (Högberg et al., 1970 Högberg & Stahle, 1971)

The biological effects of laser light at the energy levels we have used are believed to be mainly thermal, though changes due to ionization and excitation cannot be excluded (Smart, 1967 Högberg et al. 1969).

The ability to produce minute lesions within preselected regions of the inner ear is interesting from a functional point of view In the future further selective elimination of certain structures in the non-acoustic pathway or even in the organ of Corti may become possible but these advances can only be achieved if the necessary techniques are made available.

The aim of this investigation was to study the extent and reproducibility of inner-ear lesions caused by focused argon-laser irradia-

tion, including details of the hair-cell pattern in the organ of Corti. Phase-contrast microscopy and scanning electron microscopy (SEM) which complement each other were used to observe and record the findings.

MATERIAL AND METHODS

Young guinea pigs weighing about 300 g were used for this investigation. Anaesthesia was induced by intraperitoneal Nembutal and supplemented by Xylocaine injected locally into the skin. A tracheotomy was made and, if further anaesthesia was required, the administration of Penthrane by inhalation proved suitable. A special head holder fixed the animal in a supine position with the neck extended. The bulla on one side was exposed by a ventral approach for irradiation the opposite bulla was left intact as a control.

Survival times after laser irradiation varied from 2 hours to 14 days. After decapitation, both bullae were removed and opened for processing by current methods (Engström et al 1966 Marovitz et al. 1970)

A continuously operating argon laser (Coherent Radiation Laboratories, Model 52) equipped with a focusing lens and microscope, was used in all the experiments reported in this paper. The irradiation times varied between 0.5 and 30 sec at beam powers of 80 mW to 170 mW (corresponding to power densities at the focal spot of around 1 to 2 kW/cm^2) The energy is emitted at several lines in the wavelength interval 4 579–5 145 Å.

The impact point on the bony cochlea was within the dark bands caused by the pigment in the vascular stria. These bands are most easily seen near the apex of the cochlea in black-haired animals. In most of the guinea pigs the target area was the third or fourth coil from the base of the cochlea (Fig. 1). Only one point was irradiated in each animal. In a few animals the laser beam was aimed at the lateral semicircular canal. These experiments, however will be reported elsewhere.

RESULTS

The immediate effects of irradiation seen through the microscope varied from very discrete lesions in the mucous membrane and local blocking of superficial blood vessels to an obvious burn point on the otic capsule with an outer diameter of 75–100 μm (Fig. 1).

Immediately after sacrifice, the impact point of the laser beam showed in most of the animals as a minute, rounded, white-to-greyish, superficial excavation in the bony capsule. By the tenth day after irradiation this defect in the bone was covered by regenerated mucous membrane. Neither complete perforation of the bone nor a reaction of the surrounding structures in the bulla was observed in any of the irradiated animals. The extent of the outside lesion varied considerably from animal to animal at constant energy doses. This may have been due to local tissue factors influencing the energy absorption in the bone. Also it was noted in a number of animals that, if a lesion of the otic capsule was not present immediately after laser irradiation, then none will subsequently be found at sacrifice. This does not preclude, however a lesion of the organ of Corti. The extent of the inside lesions thus appears to be more directly correlated to the energy dosage

After opening the bony capsule of the cochlea, we observed an obvious tissue reaction in the vascular stria, adjacent to the point of impact on the otic capsule. This area of reaction has a dark centre surrounded by a narrow pale halo, outside of which lies a broader greyish zone, poor in detail (Fig. 2). The central zone of this irradiation response corresponds in size and position to the area of bony damage in the outer wall of the otic capsule. A reduction in the vascularity of the target area was observed.

After the vascular stria was removed to expose more fully the organ of Corti we noticed in the target area an obvious reduction in the number of osmiophilic granules in the cells of



Fig. 1. The guinea-pig cochlea observed through the microscope immediately after irradiation with an argon laser for 1 sec at a beam power of 80 mW. The impact point lies three coils from the base on the dark band caused by the pigment in the vascular stria. The damage is superficial and no perforation of the otic capsule has occurred. On the right, we have a glimpse of the tympanic membrane. ×30.

Hensen. This phenomenon was most marked in the apical region where most of the lipid granules are normally found in the guinea pig. Within a circumscribed zone the disappearance of the granules may be complete (Fig. 3). The phenomenon might be explained by a higher absorption of laser energy in the lipid drops, leading to their dissolution compared with that of the surrounding structures.

Lesions of varying character and size were found in the organ of Corti following irradiation. Cellular damage, most pronounced in the centre of the target area and subsiding peripherally, was a regular finding. Changes in the hair cells were observed to cover a wider field than is indicated by the well-demarcated entrance of the beam, clearly visible in the region of the Hensen cells (Fig. 3). De



Fig. 2 After removal of the otic capsule the impact t can be seen in the vascular stria as a round yish area with a darker centre (*arrow*). The central spot, which is surrounded by a thin light halo, corresponds to the impact zone in the bone. Blood vessels are absent within the round target area. To the right of the impact zone the Hensen cells, which contain black granules, are clearly visible. Survival time 10 days. ×35

pending mainly upon the energy dosage delivered, the size of the lesion ranged from about 10 hair cells to half of one coil. The individual hair-cell pattern within the target area observed in phase-contrast microscopy varied from complete loss to minor cytoplasmic and nuclear changes (Högberg & Stahle, 1971).

SEM, which has added a new dimension to our studies of the inner ear has a number of advantages compared with light and phase contrast microscopy in studying the post irradiation pattern. The technique gives an easily comprehensible survey as well as a wealth of detail about the surface of cellular structures (Lim, 1969 Lim & Lane, 1969 Marovitz et al., 1970 Kellerhals et al. 1970; Bredberg et al. 1970 Engström et al. 1970).

The scanning technique has revealed remarkable alterations of the surface in the centre of the target area. The predominant feature was the severe structural changes or the disappearance of the sensory hairs (Fig. 4 A, B) The most obvious damage was noted in the first row of the outer hair cells, where most of the cells had lost their cilia. In general, however the surface damage was less pronounced on the inner hair cells, as compared with the outer hair cells and, in particular those of the third row (Fig. 5). These

Fig. 3 The impact area in the third coil from the base of the organ of Corti after irradiation with an argon laser for 10 sec at 170 mW. There is complete dissolution of the dark-staining lipid granules of the Hensen cells and total loss of [illegible] µm-broad impact zone. Surface [illegible] contrast microscopy × 160.

differences in structural damage may be explained by the fact that the laser beam strikes parallel to the plane of the hair cells and consequently will affect the third row more than the other rows. The difference in reaction of the outer and inner hair cells also suggests that the latter are more resistant to damage, a finding which accords with the observations of other workers (Engström et al. 1966).

In the target area, the cilia on the outer and inner hair cells may be altered in number size and configuration. When not missing completely the common appearance was a lumping together of irregular shortened hairs (Fig. 5). On other cells, fused hairs diverge in many directions. Giant cilia formation was observed also when all th[illegible] united to form one or t[illegible] seemed longer than th[illegible] Occasionally we have s[illegible] collapsed on the surface [illegible]

Bulb-like expansion[illegible] outer hair-cell cilia w[illegible] ter irradiation. Th[illegible] mainly at the bor[illegible] where the hair-cel[illegible] was less marked. It [illegible] hair or the majority [illegible] On some cells th[illegible] rounded, on other [illegible] A further varie[illegible] cilia close to th[illegible] an olive-shaped [illegible]


A


B



Fig. 5. The same cochlea as in Fig. 4 B at higher magnification. Severe hair-cell damage after 7 days is apparent. The cilia have been raked out on some cells, while on others the remaining cilia have lost their regular pattern and have been lumped together On some outer hair cells the tops of several cilia show bulb-like expansions. 4 200.

bulb-like expansions of the tips of the hairs are not a constant feature within an irradiated area. In fact, single outer hair cells may show this unique configuration, while the neighbouring cells are almost normal in appearance. These changes do not occur to the same extent on the cilia of the inner hair cells, although "ballooning" of a few isolated cilia has been observed.

The extent of a lesion varies with the total energy dosage and the power density of the laser beam at the focal spot. Irradiation for 10 sec at 170 mW caused complete destruction of the organ of Corti over a 200-μm segment of one coil, with additional graded cellular damage in broad sectors on either side (Fig. 3). Reduction of the irradiation time to 1 sec at 170 mW restricted the lesion to about

Fig. 4 (A) Survey of the normal organ of Corti in the apical part of the cochlea, illustrated by means of scanning electron microscopy. The typical pattern with three rows of outer hair cells, one row of inner hair cells and supporting cells is apparent. 1 600. (B) Extensive lesion of the hair cells 7 days after argon-laser irradiation for 1 sec at a beam power of 80 mW. Numerous cells have lost all their cilia in other cells the remaining cilia have been seriously damaged and abnormal ciliary shapes can be discerned. Most changes have occurred amongst the outer hair cells. 1 600.



6 Bulb-like expansions of the tips of the outer cell cilia 10 days after laser irradiation for 1 at 80 mW Such expansions may be found in most cilia, as in this illustration, or in some separate cilia on an outer hair cell. 20 000.

one-third of a coil and to a more limited degree affected the organ of Corti in the coils above and below the impact zone The organ of Corti was found to be normal, however at the opposite side of the cochlea at the same level as the impact point. This indicates that the depth of penetration of laser light is limited.

With the intention of limiting the size of the lesion we reduced the power to 80 mW and the irradiation time to 0.5–1 sec in our last experiments. The extent of the damage in the organ of Corti decreased correspondingly to less than one-third of a coil and without any definite indications of hair-cell lesions in the coils apically or basally Cochleograms indicated a diminished number of missing or altered hair cells in the periphery of the target area. The "ballooning" of the tops of the outer hair cells was observed in the last series of experiments where reduced power densities were used.

DISCUSSION

The continuously acting argon laser has proved to be an instrument which can produce minute lesions of the inner ear within preselected areas. This ability to control and vary the extent of the biological lesions has been achieved by further modifications of the



Fig 7 The post-irradiation ciliary pattern on the outer hair cells may vary This figure shows an example of narrowing of the cilia, which finally expands into an olive-shaped growth. A reduction in the total number of cilia seems to have occurred. Numerous microvilli can be discerned on adjacent cells. ×9 000

length of the irradiation time, the power and the technique of accurately positioning the laser beam. The transmission of the beam can sometimes be influenced, however by individual variations in the density thickness and mineralisation of the otic capsule, which are difficult to predict (Högberg et al 1967 Högberg et al 1970) While the Q-switched ruby laser which was our first instrument (Stahle & Högberg, 1965) often had unpredictable biological effects, sometimes producing considerable lesions, the continuously acting argon laser has proved to be a more controllable instrument, mainly because close monitoring of the target area can be carried out during the irradiation period.

A feature of our last experiments was the ability to restrict the lesion to the irradiated coil alone. This finding was confirmed by an analysis of the hair-cell pattern by means of SEM. It is also worth mentioning that the change is always adjacent to the impact point the organ of Corti on the opposite side at the same level remains intact. The lesion of the organ of Corti in this last series of experiments extended over one-third of a coil or less, which was a considerable reduction, compared with our earlier experiments. As expected, the biological effect was most pronounced within the area of the central beam and subsided peripherally

A principal feature of our observations is thus the spatial limitation of the biological lesion caused by laser irradiation. This phenomenon has been noted in our previous studies on the sciatic nerve (Högberg et al., 1967) the stapes (Högberg et al., 1970) and the otic capsule of the pigeon (Högberg & Stahle, 1971) This property is valuable in experimental microsurgery and cannot be found to



6 Bulb-like expansions of the tips of the outer ..ll cilia 10 days after laser irradiation for 1 at 80 mW Such expansions may be found in most cilia, as in this illustration, or in some separate cilia on an outer hair cell. × 20 000.

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Acta Otolaryng 73 38–43 1972

ELEKTRONENMIKROSKOPISCHE UNTERSUCHUNGEN AM PLEXUS COCHLEARIS NACH KANAMYCIN INTOXIKATION

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(Eingegangen am 24 Mai, 1971)

Abstrakt. Elektronenmikroskopische Untersuchungen am Plexus cochlearis nach Kanamycin-Intoxikation lassen Strukturschäden an den Mitochondrien erkennen. Es kommt ferner zum Auftreten von stark osmiophilen Granula. Der Ribosomen-Gehalt erscheint nicht vermindert. Aus den Befunden wird auf eine Rolle des Plexus cochlearis bei der verzögerten Abgabe der Streptomyces-Antibiotika an die Perilymphe geschlossen.

Licht und elektronenmikroskopische Untersuchungen an der Cochlea nach Applikation [illegible] ototoxischen Antibiotika konzentrieren [illegible] überwiegend auf die Region der Sinnes[illegible]en (Hawkins, 1959 Beck & Krahl 1962 Duvall & Wersäll, 1964 Hawkins & Engström, 1964 Engström & Kohonen, 1965 Lundquist & Wersäll, 1966 Spoendlin 1966 Merkle et al 1968). Daneben werden noch histologisch faßbare Befunde außerhalb des Corti-Organes an Stria vascularis, Ganglion spirale und Nervus acusticus nach Kanamycin Intoxikation beschrieben. Der Plexus cochlearis (Balogh & Koburg 1965) der nach den Untersuchungen von Watanabe et al. (1969) für die verzögerte Abgabe des Kanamycins an die Endolymphe verantwortlich gemacht wird, ist dagegen auf ototoxische Schäden hin noch kaum untersucht. Lediglich die lichtmikroskopischen Befunde von Müsebeck (1965) geben gewisse Anhaltspunkte, es werden nach Kanamycin Kernvergrößerungen und Gewebsauflockerungen beschrieben. Elektronenmikroskopische Untersuchungen sollen weitere Aufschlüsse über Strukturveränderungen am Plexus cochlearis nach Kanamycin Applikation vermitteln.

Herrn Professor Dr P Falk zum 65 Geburtstag gewidmet.

MATERIAL UND METHODIK

Untersucht wurden die Schnecken von jungen Meerschweinchen mit einem durchschnittlichen Körpergewicht von 200 g 8 Tieren wurden über 21 Tage 200 mg/kg Körpergewicht Kanamycin Sulfat (Fa. Grünenthal) subcutan verabreicht. Weitere 8 Meerschweinchen dienten als Kontrollen. 2–3 Wochen nach der letzten Injektion wurden die Tiere dekapitiert und nach Freipräparation der Cochlea 4%iger eisgekühlter Glutaraldehyd in Sörensen-Phosphatpuffer (pH 7 4) entlang dem Nervenstamm und nach Durchstechen der Schneckenspitze in die Basalwindung injiziert. Der Modiolus wurde anschließend völlig freipräpariert und verblieb für 8 Stunden in 4%igem Glutaraldehyd Es erfolgte dann 14-tägiges Entkalken bei 0 C in einer 0 1 m *EDTA* Lösung (eingestellt auf pH 7 4 mit NaOH) in der oben angegebenen Fixationslösung. Nachfixation für jeweils 2 Stunden in 1%iger Osmiumsäure in Veronal-Acetat-Puffer (pH 7 4) mit Zusatz von 4,5% Saccharose und 3%



Abb. 1. Plexus cochlearis der Meerschweinchencochlea. Die Plexuskapillaren (*C*) sind von Zellen mit feinen und meist parallel verlaufenden Lamellen umgeben. *N* Zellkern. *E*, Erythrocyt. Vergr. 16 000fach.

igem Formaldehyd in Sörensen-Phosphatpuffer (pH 7.4) (Baird et al., 1967). Nach Araldit einbettung wurden die Ultradünnschnitte (Ultramikrotom Om U2, Fa. Reichert) mit Uranylacetat (Watson, 1958) und Bleicitrat (Reynolds, 1963) kontrastiert. Die elektronenmikroskopischen Aufnahmen wurden mit dem Siemens Elmiskop I bei einer Primärvergrößerung von 8 000 hergestellt.

ERGEBNISSE

Der Plexus cochlearis, der die Gefäße des Tractus spiralis arteriosus et venosus begleitet, besteht elektronenmikroskopisch aus Zellen mit langgestreckten und parallel ausgerichteten faltenartigen Lamellen (Abb. 1). Das Cytoplasma enthält zahlreiche Mitochondrien, ein relativ gut ausgeprägtes granuliertes endoplasmatisches Retikulum sowie Lysosomen. Nicht selten kommen Polysomen zur Darstellung. Auffällig sind intracelluläre Vakuolen unterschiedlicher Größe, wobei die kleineren in unmittelbarer Nachbarschaft der Golgizone liegen. Von diesem Gewebe werden die zahlreichen Plexuskapillaren umschlossen. Diese besitzen ein geschlossenes Endothel, das von einer dünnen und mäßig elektronendichten Basalmembran umgeben ist. Falten wölben sich in das Gefäßlumen vor. Pericyten kommen gelegentlich vor (Mootz & Milsebeck, 1970; Mootz & Schöndorf, 1971).

Nach Verabreichung von 21 × 200 mg Kanamycin-Sulfat treten degenerative Veränderungen an den Mitochondrien auf. Es kommt dabei zu Deformationen und Verklumpungen der *Cristae mitochondriales*. *Myelinfiguren* beobachtet man in Form von unregelmäßig verlaufenden Lamellen oder als mehr konzentrische Figuren, die einen großen Teil des Mitochondriums ausfüllen können. Erste Stadien dieser Veränderungen scheinen Schwellung und Aufhellung der Struktur an einigen Mitochondrien zu sein. Im Endstadium sind keine Myelinfiguren erkennbar. Statt dessen kommen Vakuolen zur Darstellung, die zum Teil mit amorphem osmiophilem Material angefüllt sind (Abb. 2, 3). Diese Veränderungen betreffen jedoch meist nur einen Teil der Mitochondrien, während andere völ-



Abb 2 Plexus cochlearis nach 21 × 200 mg/kg Kanamycin. Mehrere Mitochondrien mit degenerativen Änderungen (→) *N* Zellkern. Vergr 23 400fach

ig intakt aussehen. Die übrigen Zellorganellen scheinen nicht geschädigt zu sein. In Kernnähe liegen öfter lysosomale Elemente mit osmiophilen Einschlüssen, die gegenüber den unbehandelten Kontrollen größer sind und zahlreicher vorkommen (Abb 3)

DISKUSSION

Die von uns nach Kanamycin-Intoxikation beobachteten Veränderungen an den Mitochondrien der perikapillären Zellen des Plexus cochlearis gleichen denen die Duvall & Wersäll (1964) nach Streptomycin Applikation an den Sinneszellen des Corti-Organs nachweisen konnten. Danach wird das Membransystem der Mitochondrien desintegriert, es kommt zum Auftreten von eigenartigen konzentrischen Lamellen sowie zur Vakuolisierung. Die Zellfunktion scheint jedoch nicht zum Erliegen zu kommen, da nicht alle Mitochondrien derartige Veränderungen aufweisen und manche Zellen überhaupt nicht geschädigt sind. Da die Mitochondrien Träger von wichtigen Enzymen für den Zellstoffwechsel sind, ist daher bei der Interpretation unserer Befunde am ehesten daran zu denken daß einzelne Stoffwechselsysteme blockiert werden (Friedmann & Bird, 1961 Mascitelli-Coriandoli, 1962) Das Vorkommen von osmiophilen Granula wurde von Wersäll & Hawkins (1962) nach Streptomycin-Intoxikation in den vestibulären Sinneszellen beschrieben, ebenso von Spoendlin (1958) nach akustischer Belastung in den Haarzellen des Corti-Organs. Es wäre daran zu denken, daß in einigen Fällen auch Reste von untergegangenen Mitochondrien darin enthalten sind, zumal man gelegentlich



Abb. 3 Mitochondrien mit Myelinfiguren (→). Lysosomen mit osmiophilen Granula (*L*) in der Nähe des Zellkernes (*N*). Vergr. 24 600fach.

membranartige Strukturen in der Matrix zu erkennen glaubt.

Die Ototoxizität ist auf einen Konzentrationseffekt zurückzuführen (Stupp et al., 1966). Die hierdurch bedingte Kanamycinanhäufung und außergewöhnlich lange Einwirkungszeit im Innenohr und danach für die spezifisch ototoxische Wirkung ursächlich verantwortlich. Peri- und Endolymphe enthalten wesentlich höhere Konzentrationen der Streptomyces-Antibiotika als Blut und Liquor. Dabei erfolgt die Anreicherung des Kanamycins im Innenohr deutlich langsamer als im Blut, sein Abtransport aus dem Innenohr ist wesentlich verzögert und die Resorption erschwert. Diese beiden Fakten werden dadurch erklärt, daß die ototoxischen Antibiotika nicht resorbierbar sind. Ungeklärt bleibt noch, weshalb es zu einem solchen Konzentrationsgefälle kommt. Naheliegend ist es, daß sich die primären pathologischen Vorgänge im „perilymphatischen Gewebe" abspielen. So wird von Stupp et al. (1966) die Stria vascularis ursächlich für die lokale Kanamycinanreicherung im Innenohr verantwortlich gemacht. Auch konnten Beck & Krahl (1962) Zellschäden an der Stria vascularis nach Kanamycin-Intoxikation nachweisen. Mißebeck & Schätzle (1964) beobachteten eine Reaktionsabschwächung der schwach sauren Mucopolysaccharide vom Typ des Heparins im Ligamentum spirale und im Limbus spiralis nach Dihydrostreptomycin, die sie als Retention durch Bindung der basischen Aminoglykoside an saure Mucopolysaccharide ansehen. Die Einbeziehung des Plexus cochlearis in den ototoxischen Prozeß weist erneut darauf hin, daß sich die Schädigung keineswegs auf die im Vordergrund der Untersuchungen stehenden Sinneszellen beschränkt, sondern auch

das resorbierende und sezernierende „perilymphatische Gewebe" erfaßt. Dem Plexus cochlearis dürfte bei dem Vorgang der Kanamycin-Anreicherung im Innenohr keine unbedeutende Rolle zufallen, wobei an seiner sekretorischen Leistung nicht länger gezweifelt werden darf (Balogh & Koburg 1965; Müsebeck, 1965; Schätzle & von Westernhagen, 1968; Mootz & Müsebeck 1970; Mootz & Schöndorf 1971). In seiner engen topographischen Beziehung zu den Gefäßen des *Tractus spiralis arteriosus et venosus* und wegen seines Kapillarreichtums könnte dieses Modiolusgewebe sogar den bevorzugten Ort der Perilymphproduktion darstellen (Schreiner 1965; Koburg, 1965), zumal bekannt ist, daß die Perilymphe ein Ultrafiltrat des Serums ist (Schindler 1965). Watanabe et al. (1969) ziehen aus der Tatsache, daß die Kanamycin-Spiegel in der Endolymphe zeitlich denen im Serum und in der Perilymphe nachhinken, den Schluß, daß das Kanamycin vom Plexus cochlearis in die Perilymphe abgegeben wird. Der *Stria vascularis* wird hierbei keine Bedeutung zugemessen. Erst 24 Stunden später ... in das Kanamycin in der Endolymphe ... hgewiesen werden, wo es dann schließlich in einer Sackgasse liegenzubleiben scheint (Stupp et al., 1966). Unsere Befunde schließen die Möglichkeit ein, daß auch der Abtransport von Kanamycin aus der Peri- und Endolymphe durch Schädigung des Plexus cochlearis verzögert vor sich geht.

SUMMARY

Examination of the plexus cochlearis with the electron microscope showed structural damage of the mitochondria as a result of kanamycin-intoxication. Intensive osmiophile granules could also be observed. The number of ribosomes was not diminished. It may be suggested that as a result of damage to the plexus cochlearis, the excretion of streptomyces-antibiotics into the perilymph was retarded.

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Acta Otolaryng 73 44–52, 1972

DIRECTIONAL AUDIOMETRY

II The Influence of Azimuth on the Perception of Speech in Aided and Unaided Patients with Monaural Hearing Loss

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(Received February 10, 1971)

Abstract In a group of 20 patients with monaural hearing loss varying from a Pure Tone Average (PTA) of 20 dB hearing level to residual hearing, the Directional Threshold of Intelligibility (DTI) was measured both with and without background noise, and with and without hearing aid.

Without background noise, the DTI with hearing aid was found to be better than the DTI without hearing aid in [illegible] of the 4 positions of the signal loudspeaker The difference was statistically significant. With [illegible]ground noise, the DTI with hearing aid was [illegible] to be significantly better than without hear[illegible] aid in 6 of the 16 combinations of signal and [illegible]e loudspeakers. For all of the 20 experimental [illegible]ening conditions we found that the DTI was better with hearing aid than without in 11 of the 20 patients. For the 16 experimental listening conditions with background noise we found a significantly better DTI in 9 of the 20 patients, in [illegible] patients we found a poorer DTI with hearing aid than without.

It seems important to evaluate the effect of hearing aid treatment of monaurally hard-of-hearing patients after a sufficiently long trial period

It is accepted that persons with monaural hearing impairment are not to be considered as normally hearing subjects (Wark, 1967 Giolas & Wark 1967) Malles (1963), considered, after his own investigations, that hearing-aid treatment is desirable in patients with monaural hearing loss of 30 dB or more and Harford & Musket (1964) reported good results from treating some patients in this way In a case of unilateral conductive hearing loss and normal hearing in the other ear Miller & Lehman (1969) used a hearing aid with bone conductor

In previous publications the apparatus for the measurement of the Directional Threshold of Intelligibility (DTI) (Tonning, 1970) and the definition of the DTI (Tonning *a* in press), have been described. Analysis of the DTI values found in a group of 37 patients having various degrees of monaural hearing loss has been reported (Tonning *b* in press)

From an audiological point of view patients with monaural hearing loss can be divided into two sub-groups, according to the type of treatment which seems indicated.

A. Patients having a not too severe monaural hearing loss may be expected to receive benefit from a hearing aid on the bad ear

B Patients having a very severe monaural loss of hearing, cannot be expected to benefit from a hearing aid on the bad ear Under certain conditions, however it seems probable that a crossover hearing aid which could route signals from the hard-of-hearing side of the patient's head to the good ear might be helpful.

This publication is concerned with the type of patients described in paragraph A above. In the experiment reported here 20 patients belonging to the group A were examined.

Table I *The relationship between British Standard 1954 and ISO Standard 1964 for our equipment (earphone TDH 39/MX41AR 9A coupler)*

The table indicates the number of dB that must be added to the threshold of hearing recorded in British Standard when transferring to ISO Standard

Frequency Hz	125	250	500	1000	000	4 000	6 000	8 000
dB added to hearing loss in British Standard when transferring to ISO Standard	+3.5	+3.7	+2.0	+2.9	−0.2	0.0	+7.9	+5.5

Using the previously described technique, the DTI values both with and without hearing aid were recorded.

METHOD

Throughout the following, the dB reference level is 0 0002 dyne per square centimetre Hearing loss in dB refers to British Standard 2497 1954 The relationship between ISO-Standard 1964 and British Standard 1954 for our equipment is shown in Table I (see also Whittle & Delany 1966).

The Directional Threshold of Intelligibility (DTI) was recorded with the signal loudspeaker reproducing speech in four different positions in the horizontal plane (0 − in front, 90° − to the right, 180 − behind, and 270 − on the left side of the person tested) A series of four tests without background noise were made on each person tested. The experiment was then made against background noise (65 dB white noise) for each of the four positions of the loudspeaker reproducing speech, four positions (0 90 180 and 270) of the white noise source were used, giving 16 different combinations of positions of the loudspeakers. The dB value of DTI refers to the intensity at the point 0.9 metre from the signal loudspeaker This point corresponds to the centre of the head of the person tested. This value was controlled by calibrating the equipment without any person in the sound field prior to the testing. The intensity of the white noise was likewise adjusted to 65 dB at this central point.

The DTI values of each person were first determined without the use of a hearing aid. Prior to the tests with the hearing aid, the volume control of the aid was adjusted to an appropriate level by means of conversation with the operator under conditions being like for all the patients tested.

MATERIAL

20 right-handed experimental subjects (5 women and 15 men) from 12 to 68 years old (average age 44 years, median age 43.5 years), were examined. Pure tone audiograms were recorded for the frequencies 125 250 500 1 000 2 000 4 000 6 000, and 8 000 Hz, using a Madsen Electronics Audiometer Model OB 60 calibrated according to British Standards. The hearing loss occurred in the right ear in 10 cases, and in the left ear in the remaining 10 cases. In order to avoid extremely steep audiogram curves, only subjects with an individual difference of 30 dB or less between the best and poorest threshold values at 500 1 000, and 2 000 Hz in the bad ear were accepted. All the good ears had a PTA less than 20 dB hearing level. (In this paper the hearing loss is given as PTA, i.e. Pure Tone Average the mean of the hearing levels at the frequencies 500, 1 000, and 2 000 Hz.)

The patients had used hearing aid for at least 3 months before the tests with hearing aid were made. Eight of the patients (nos. 2,

Table II *Patients with unilateral hearing loss Patients 1–10 have impaired hearing in the right ear nos 11–20 in the left ear*

PTA Pure Tone Average: Mean of the hearing levels at 500, 1000 and 2000 Hz in the bad ear
Re Residual hearing. Hearing not measurable at all frequencies tested.
Cond Conductive hearing loss.
Sn. Sensorineural hearing loss.
Mixed Combination of conductive and sensorineural hearing loss.
Fowler test + indicates recruitment – indicates no recruitment. No sign indicates that the test has not been done

No.	PTA	Type hearing loss	Fowler test	Sex	Age	Hearing aid
1	30	Mixed	–	♂	55	Aditone 620
2	33	Sn	–	♀	53	Widex 641
3	36	Mixed	–	♀	62	Oticon 560 S
4	45	Mixed	–	♂	32	Oticon 830 S
5	45	Cond		♂	23	Oticon 830 S
6	45	Mixed	–	♂	39	Oticon 560 S
7	55	Sn	+	♂	19	Otwidan 651 E12
8	55	Mixed	–	♀	44	Otwidan 651 E12
9	83	Mixed		♂	68	Aditone 655 PP
10	85	Sn		♂	43	Oticon 560 PP
11	32	Sn	+	♂	61	Aditone 620
12	47	Cond		♀	47	Otwidan 651 E12
13	53	Sn	+	♂	36	Otwidan 651 E12
14	53	Sn	+	♂	61	Otwidan 651 E12
15	55	Mixed	–	♂	42	Otwidan 651 E12
16	60	Sn	+	♂	12	Otwidan 652 E12
17	60	Sn	–	♂	53	Otwidan 652 E12
	65	Mixed	–	♀	37	Otwidan 652 E12
	80	Mixed		♂	59	Aditone 655 PP
	Re	Sn		♀	35	Oticon 560 PP

5 6 8 11 15 16 and 18) had used other hearing aids before this investigation started.

In Table II the patients are listed in two columns with increasing hearing-loss in the right or left ear respectively

RESULTS

DTI without noise both without and with hearing aid

The DTI values without and with hearing aid were recorded for the various positions of the signal loudspeaker The mean values for DTI ($\overline{DTI}$) were calculated for the four different positions of the signal loudspeaker and listed in Table III.

Wilcoxon-Test for paired comparisons was

Table III *The influence of azimuth on the aided and unaided speech reception thresholds in 20 monaural hard-of-hearing subjects in quiet*

$\overline{DTI}$ The mean value of DTI for each position of the signal loudspeaker

Position of signal loudspeaker	DTI without hearing aid	DTI with hearing aid	Effect of hearing aid on DTI
In front	22.6	21.7	No effect
Bad ear	25.4	22.7	Improvement
Behind	23.4	20.7	Improvement
Good ear	20.3	19.5	No effect

used, level of significance 0.05 (Statistics in this article according to Dixon & Massey 1957 Siegel, 1956. Electronic computer was used, the programs being taken from IBM's System 360 Scientific Subroutine Package (360A-CM-03X) Version III 1968.)

It was found that the DTI with hearing aid was statistically significant better than the DTI without hearing aid when the signal loudspeaker was in the positions 1) aiming directly at the pathological ear or 2) behind the patient.

DTI in noise both without and with hearing aid

In this experimental series, DTI was determined during simultaneous stimulation with noise. The signal loudspeaker was placed in each of the previously mentioned four positions in turn. For each of these positions, the noise loudspeaker was placed in the same four positions, resulting in a total of 16 different combinations of positions. For each of the 16 combinations of signal and noise loudspeakers the arithmetic means of the DTI values ($\overline{DTI}$) were calculated both without and with hearing aid, see Table IV

Using Wilcoxon Test again, the DTI values for each patient were analysed to determine whether there was significant difference or not between the values found without and with the use of hearing aid under the

Table IV *The DTI values (= the arithmetic mean of the DTI values) for the various combinations of signal and noise loudspeakers both without and with the use of hearing aid*

Position of signal loudspeaker	DTI without hearing aid	DTI with hearing aid	DTI without hearing aid	DTI with hearing aid
	Position of white noise loudspeaker: In front		Position of white noise loudspeaker: Behind	
In front	53.5	51.7	51.4	51.1
Bad ear	53.6	51.4	53.0	52.5
Behind	52.7	51.1	52.9	52.5
Good ear	49.4	48.9	48.2	48.4
	Position of white noise loudspeaker: Bad ear		Position of white noise loudspeaker: Good ear	
In front	49.[illegible]	49.6	53.5	53.2
Bad ear	54.3	53.1	54.9	51.8
Behind	50.5	50.4	54.7	52.5
Good ear	47.3	47.2	52.9	52.7

16 test conditions. The results of these calculations are listed in Table V

From Table V it can be seen that the DTI is significantly improved by the use of hearing aids in 6 of the 16 experimental conditions.

It should be noted here that, with the exception of the patients 1 and 11 all of the subjects are fitted with hearing aids having the microphones located either above the auricle (nos. 4 and 5) or behind the auricle (the remaining subjects). This material does not, however permit an evaluation of the significance of the microphone position.

Regarding the results for the persons with right-sided hearing loss and the persons with left-sided hearing loss separately we observed that the best listening positions without hearing aid corresponded for these two populations, see Fig. 1 The poorest and the intermediate listening positions were nearly the same for the two groups.

With hearing aid the differences between the best, the intermediate and the poorest listening positions were more marked, and the effect of hearing aid was not found to be the same for all the corresponding loudspeaker positions for the two groups (Fig. 1)

Fig 1 seems to indicate that our patients are not to be regarded as a homogeneous population as far as hearing is concerned. Hearing aid may be expected to have different effects on different patients, and this we have tried to investigate

Table V *The effect of hearing aid on the DTI for the 16 various combinations of signal and noise loudspeakers*

Position of signal loudspeaker	Position of the white noise loudspeaker: In front	Bad ear	Behind	Good ear
In front	Improvement	No effect	No effect	No effect
Bad ear	Improvement	Improvement	No effect	Improvement
Behind	Improvement	No effect	No effect	Improvement
Good ear	No effect	No effect	No effect	No effect

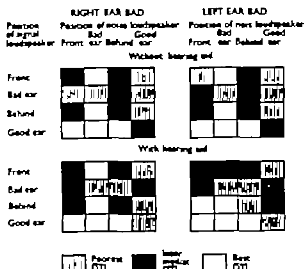


Fig 1 Diagram showing the 5 poorest, the 6 intermediate, and the 5 best listening conditions against background noise. 10 persons with right-sided and 10 persons with left-sided hearing loss are tested without and with hearing aid.

Let us consider two experimental listening conditions: A and B

Condition A The signal loudspeaker aim at the bad ear the noise loudspeaker aim at the good ear

Condition B Both loudspeakers facing the bad ear

If the bad ear is of importance for the discrimination of speech in noise we would expect lower (better) DTI values under condition A than under condition B Subtraction of the second mentioned DTI value (DTI B) from the first (DTI A) would then result in a negative value. If the bad ear is of no essential importance for the discrimination of speech in noise we would expect lower (better) DTI values under condition B than A and the difference DTI A − DTI B would be a positive.

It has previously been demonstrated (Tonning *b* in press) that in the white noise of 65 dB in certain cases under our experimental conditions, a bad ear could perceive speech if the monaural hearing loss in this ear was PTA 53 dB hearing level or less, i.e. with increasing monaural hearing loss the difference DTI A − DTI B shifts from negative to positive values at a level of PTA 53–55 dB hearing level.

In order to find out whether hearing aid can move this level, the observed DTI values without hearing aid for these two listening conditions A and B and the differences between these values, are listed on the left side in Table VI.

Here we find that the previously described shift from negative to positive values without hearing aid occurs at a hearing loss corresponding to PTA 53–55 dB hearing level (Tonning *b* in press) This implies that under the described conditions the pathological ear was functioning as a receptor of speech if the hearing loss was not greater than PTA 53 dB hearing level.

In the same way the observed DTI values measured with the use of hearing aid are tabulated to the right in Table VI The calculations DTI A − DTI B are now performed using these figures, and negative differences are found up to PTA 65 dB hearing level. This implies that under the described experimental conditions the pathological ear by means of the hearing aid contributed to the perception of speech if the hearing loss did not exceed PTA 65 dB hearing level. In this respect it should be pointed out that.

1 The figures listed in Table VI and the conclusions drawn from them must be treated with great caution, due to the fact that the deviation of the individual values has not been taken into consideration.

2 Patient 7 displays positive values both with and without the use of a hearing aid. This point will be discussed below

3 The present subject group does not permit an accurate determination of the level at which the above-mentioned differences shift from negative to positive values, due to the fact that we have no patients in our group between PTA 65 dB and PTA 80 dB hearing level.

Table VI. *Figures showing the influence of the hearing aid on DTI values under certain conditions*

DTI A indicates the DTI measured with the signal loudspeaker aimed at the bad ear and the noise loudspeaker on the opposite side, aimed at the good ear. *DTI B indicates the DTI measured with both loudspeakers facing the bad ear*

Pat. no.	PTA of bad ear	Without hearing aid			With hearing aid		
		DTI A	DTI B	DTI A DTI B	DTI A	DTI B	DTI A–DTI B
1	30	51	56	−5	50	54	−4
11	32	48	55	−7	47	54	−7
2	33	52	54	−2	46	55	−9
3	36	55	55	0	48	54	−6
4	45	58	61	−3	54	56	−2
5	45	54	55	−1	51	51	0
6	45	52	55	−3	51	51	0
12	47	51	52	−1	47	53	−6
13	53	51	53	−2	57	52	0
14	53	55	57	−2	52	52	0
15	55	55	52	+3	51	51	0
7	55	55	49	+3	55	54	−1
8	55	58	54	+4	52	53	−1
16	60	57	53	+4	52	53	−1
17	60	55	53	+1	50	50	0
18	65	57	56	+1	53	54	−1
19	80	58	52	+6	56	51	+5
9	83	65	55	+10	55	54	+1
10	85	57	56	+1	57	55	+2
20	Re	56	52	+4	56	54	+2

The effect of hearing aid on the individual DTI

Using Wilcoxon Test for paired comparisons, level of significance 0.05 we have for each patient calculated whether the hearing aid affected

1. the DTI for all of the 20 experimental set ups (DTI_{20}) 4 loudspeaker positions without background noise and 16 positions with background noise.

2. the DTI for the 16 listening conditions with background noise (DTI_{16}).

The results of these calculations are shown in Table VII. We find that DTI_{20} for 11 of the 20 patients is better with hearing aid than without. For none of the 20 patients was DTI_{20} found to be worse with hearing aid.

For 9 of the 20 patients the DTI_{16} is better with hearing aid than without, for 9 there is no improvement. For 2 patients, nos. 7 and 11 the DTI_{16} is worse with hearing aid.

Concerning patients 7 and 11 the achievement of a worse DTI_{16} value with the use of a hearing aid than without might be due to specific conditions in the individual patient. Patient 7 showed recruitment, and had a continuous tinnitus. The annoyance of the tinnitus was not affected by the hearing aid and the background noise was much more disturbing with the hearing aid than without. Patient 11 also had recruitment and tinnitus in the pathological ear. The tinnitus was subjectively reduced by the hearing aid, but background noise caused the speech to blend to a continuous mumble.

Use of the standard diagram

In evaluating the effectiveness of the hearing aids, the standard diagram previously described (Tonning, *a*, and *b* in press) may be used. Referring to Fig. 3 the abscissa axis indicates values of DTI without noise. The ordinate axis indicates values of DTI with noise divided by the corresponding DTI without noise i.e. with the signal loudspeaker in the same position. In this coordinate system a figur

been drawn, covering the area where 95% of the DTI values of the 30 normally hearing subjects described earlier can be plotted. In order to make the diagram more instructive, and to avoid the trouble of calculating the fraction

$$\frac{\text{DTI with noise}}{\text{DTI without noise}}$$

five line segments plotting some of the most common values of DTI observed with noise namely DTI = 40 45 50 55 and 60 dB, have been drawn.

In Fig. 2 the standard diagram is used to illustrate how hearing aid can influence the DTI values under various experimental listening conditions. DTI values of 2 patients with impaired hearing in their left ears are plotted in the diagram.

Patient 17

1 Without hearing aid, the signal loud speaker aimed at the left ear the noise loudspeaker aimed at the right ear

DTI without noise

dB

1 with noise

55 dB

Point plotted at A.

2. With hearing aid, the same positions of the loudspeakers:

DTI without noise

27 dB

DTI with noise

50 dB

Point plotted at B

Point B lies further to the left in the standard diagram and is closer to the area of the normally hearing 30 standard subjects, while it also lies on an iso-line further down in the coordinate system than point A. These factors indicate that under condition B (with hearing aid) the DTI values in both silence and noise are better than under condition A (without hearing aid). Point A and point B lie on iso-

Table VII. *The effect of hearing aid for each of the 20 patients*

DTI_{20}. The DTI for each patient both with and without background noise (4 loudspeaker positions without, and 16 with background noise)

DTI_{16}. The DTI for each patient with background noise (16 loudspeaker positions)

Pat. no	The effect of hearing aid on	
	DTI_{20}	DTI_{16}
1	Improvement	Improvement
2	No effect	No effect
3	Improvement	Improvement
4	Improvement	Improvement
5	Improvemnet	Improvement
6	Improvement	Improvement
7	No effect	Impairment
8	Improvement	Improvement
9	Improvement	Improvement
10	No effect	No effect
11	No effect	Impairment
12	Improvement	No effect
13	Improvement	No effect
14	Improvement	Improvement
15	No effect	No effect
16	No effect	No effect
17	Improvement	Improvement
18	No effect	No effect
19	No effect	No effect
20	No effect	No effect

lines going through the area of the 30 normally hearing subjects, indicating that the DTI in noise both with and without hearing aid are not worse than the DTI values of the normally hearing subjects. However the fact that the points A and B lie to the right of the area of the normal hearing standard group indicates that in silence the DTI values of patient 17 are poorer than the corresponding DTI values of the 30 normally hearing persons. This is also the case when a hearing aid is used.

Patient 20

1 Without hearing aid signal loudspeaker aimed at the right ear noise loudspeaker aimed at the left ear

DTI without noise

16 dB

DTI with noise

43 dB

Point plotted at C.

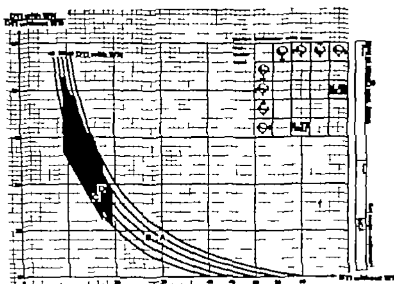


Fig 2 Coordinate system, illustrating the effect of monaural hearing aid under certain conditions on 2 patients (nos. 17 and 20) with impaired hearing in the left ear For further explanation see text.

2. With hearing aid, the loudspeakers in the same positions:
DTI without noise
17 dB.
DTI with noise:
49 dB
Point plotted at D

Points C and D both lie in the area covering 95% of the DTI values for the 30 normally hearing standard persons, indicating that the DTI values of patient 20 both without and with hearing aid in silence and in noise do not differ from the corresponding DTI values of the standard group. However point D lies further to the right than point C, and point D lies on an iso-line further up in the *coordinate system than point C. This indicates* that under condition D (with hearing aid) the DTI values in both silence and noise are worse than under condition C (without hearing aid).

When analysing these factors, it must be remembered that the deviations in the results of the measurements are not taken into consideration. If this is remembered together with the fact that the positions of the speakers relative to each other and to the patient also influence the results, it should be possible to get an impression of the effect of the hearing aid on the DTI under certain conditions, by using the standard diagram. This impression *could then be of help in making an evaluation* of the effectiveness of the hearing aid treatment.

COMMENTS

It is difficult to state the upper and lower limits of a monaural hearing loss within which a hearing aid may be of benefit as many variable factors are involved. It should be noted that both the patient's employment and subjective attitudes towards the hearing loss are of significance. Furthermore even though the hearing loss may seem quite insignificant, *hearing aid treatment may be indicated by the fact* that it sometimes subjectively seems to dampen tinnitus slightly (patient 11) or completely (patients 2, 4 and 14). Patient 15 had a feeling of auditory imbalance in the head without hearing aid. This first became clearly apparent after the hearing aid treatment was begun, so that it cannot be denied that this might be a byproduct of the hearing aid treatment itself

Patients 4 and 13 also found the hearing aid treatment comfortable because of the fact that they no longer needed constantly to turn the head when conversing with more than one person.

It is clear that the degree of hearing loss is not the sole factor involved for satisfactory results of treatment with a hearing aid. On the basis of the observations which we have recorded here, we would under our experimental conditions expect some effect from head borne hearing aid when the patient had a monaural PTA less than 80 dB hearing level. It must be stressed that our experimental listening situation differed considerably from a real hearing situation in everyday life the investigations were made in an anechoic room. The degree of intensity of the noise employed was not out of the ordinary the character of the noise, however was not of common occurrence. The investigation of the speech perception took place at the threshold. The effectiveness of the hearing aid treatment has therefore to be evaluated after a trial period, and the patient's own experience and impression of the hearing aid treatment must taken into account.

This paper deals with the measurable effect of a hearing aid under certain experimental listening conditions on patients with monaural hearing loss. The different questions of which patients are motivated for such treatment and which really carry through the treatment fall outside the scope of this publication.

ZUSAMMENFASSUNG

In einer Gruppe von 20 Patienten mit einseitigen Hörschäden, welche von einem Reinton-Durchschnitt (PTA = Pure Tone Average) von 20 dB bis zu zurückbleibendem Hörvermögen variierten, wurde die gerichtete Verständlichkeitsschwelle (DTI = Directional Threshold of Intelligibility) sowohl mit als auch ohne Hörhilfe gemessen.

Ohne Hintergrundgeräusch fand man die DTI mit Hörhilfe statistisch signifikant besser als DTI ohne Hörhilfe in ? der 4 Positionen des Signallautsprechers. Mit Hintergrundgeräusch fand man die DTI mit Hörhilfe statistisch signifikant besser als ohne Hörhilfe in 6 der 16 Kombinationen von Signal- und Geräuschlautsprechern. Für sämtliche 20 Versuchs-Hörverhältnisse fanden wir bessere DTI mit Hörhilfe als ohne für 11 der 20 Patienten. Für die 16 Versuchs-Hörverhältnisse mit Hintergrundgeräusch fanden wir bei 9 von den 20 Patienten signifikant bessere DTI, und für 2 von ihnen fanden wir schlechtere DTI mit Hörhilfe als ohne.

Es scheint wichtig, die Wirkung von Hörhilfenbehandlung von Patienten mit einseitigen Hörschäden nach einer genügend langen Probezeit zu bewerten.

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Acta Otolaryng 73 53–60 1972

TYMPANOPLASTY IN CHRONIC ADHESIVE OTITIS MEDIA

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(Received May 12, 1971)

Abstract. The results of tympanoplasty on 56 patients with chronic adhesive otitis media are presented. The principle of the method was radical removal of the diseased parts of the drum, preservation of the mucosa, inspection and opening of the tubal orifice with bougienage of the tube mobilization of the ossicles, ossiculoplasties increasing the height of the tympanic cavity insertion of silastic film around the stapes, and tubulation. The mean hearing gain in the frequency range 500–2 000 cps 3–9 months after the operation was 23.5 dB post-operative hearing 35 dB and air-bone gap 15 6 dB. In 80% of the cases the tympanoplasty proved successful, and 86% of the patients obtained a hearing gain exceeding 10 dB. The most favourable results were observed in cases tubulated at the time of the tympanoplasty The results are analysed in relation to tubal passage, assessed by the Valsalva manoeuvre before and after the operation. The best results were obtained when the tube was passable before and after the operation in the Valsalva manoeuvre, poorest when it was not passable either before or after the operation.

Chronic adhesive otitis which is the terminal stage of chronic secretory otitis, presents the most difficult problems in tympanoplasty (Wullstein, 1968) As yet, there have been but a few reports on the postoperative results which are on the whole not as good as in sequelae of otitis. The condition is common. Classifying 35 000 patients with hearing impairment in the Copenhagen Hearing Centre Ewertsen (1963) found that 10% of the cases with conduction deafness were due to chronic adhesive otitis media and 13% to otosclerosis. Since adhesive otitis is frequently bilateral, it presents an important field to the otosurgeon, as the patient may derive great benefit from an ever so slight permanent hearing gain after the tympanoplasty

In recent years, secretory otitis has been on the increase, and although draining of the middle ear (Armstrong, 1954) in the early stages has afforded good results, there does not seem to have been any perceptible decrease in the incidence of adhesive otitis (Siirala & Kohonen, 1970). Despite great efforts, including repeated tubulations, an ever larger number of cases of chronic secretory otitis are ending in the adhesive stage.

Siirala (1964 *b*) has reviewed the older methods of treating adhesive otitis. Recently implantation of mucous membrane from the maxillary antrum and lip to the tympanic cavity (Wullstein, 1963 Zöllner 1963) and filling of the tympanic cavity with paraffin (Rambo 1961) have been tried. However the results have not been convincing, and these methods have been largely abandoned in favour of inserting silastic film into the tympanic cavity (House & Sheehy 1963 Gerhardt, 1968). Siirala (1964 *b*) has treated adhesive otitis by mastoidectomy and freeing of the retracted drum, removal of adhesions and mucous membrane from the tympanic cavity to the tubal orifice, whence re-epithelialization was supposed to start. The middle ear was regularly filled with corticosteroids and chemotrypsin and ventilated by a polyethylene tube passed from the tympanic cavity by way of the Eustachian tube and the nose (Zöllner

1963) or by way of the retroauricular mastoidectomy wound

By operation on 13 cases of adhesive otitis Zöllner (1963) obtained social hearing in 7 Operating upon 43 ears in the adhesive stage Siirala (1964 *a*) obtained a hearing improvement exceeding 10 dB in 33 cases 14 patients obtained social hearing. In another series of 25 cases in the adhesive stage operated upon by the same principles (Siirala, 1964 *b*) the result was social hearing in 11 and a hearing gain exceeding 10 dB in 18. Gerhardt (1968) obtained social hearing in 10 out of 22 operated cases with adhesive otitis. In most of the cases, however a radical cavity was transformed to type IV tympanoplasty Out of 14 operated cases Ekvall (1969) reported social hearing in 8 and a hearing improvement exceeding 10 dB in all.

Surgical technique and postoperative management

Our principle in tympanoplasty has been radical removal of the diseased parts of the drum, ...ring them with fascia, preservation of as ...1 mucosa as possible, even though it is ...ted, inspection and opening of the tubal ...ifice with bougienage of the tube mobilization of the ossicles and the use of ossiculoplasties which enlarge the tympanic cavity Moreover we use tubulation and small pieces of silastic film, especially around the stapes Otherwise the tympanoplastic procedures do not differ essentially from those used in chronic otitis (Zöllner 1966 Wullstein 1968) except that regard was paid to the special problems presented by adhesive otitis

(1) *The drum* This was always retracted either *in toto* or in major or minor areas. As a rule, it was thin, inelastic, and adhering to the promontory Posteriorly and around the niches the drum was most retracted imitating a pseudoperforation or a cholesteatoma membrane. In this membrane there might be a genuine perforation, leaving the mucosa exposed. Even after complete detachment, such a drum is useless. It has lost its elasticity it is too large and is bound to retract again soon *Therefore, the entire thin drum as well* as the adherent membrane were removed, often leaving total or subtotal perforation. In those places, especially at the edges, where the drum was of normal thickness and elasticity and non-adherent, it was preserved, but de epithelialized. In particular it is attempted to preserve the drum between the handle of the malleus and the anterior fold of the malleus in order to insert a tube in this site.

In some cases the drum as a whole was diffusely thickened, stiff and immovable. In such cases parts of the drum were removed, others thinned by removing squamous epithelium and the fibrous or tympanosclerotic layer

(2) *Tympanic cavity* The changes in the tympanic cavity might be diverse but an invariable finding is a thickened mucosa, marked adhesions, and a diminished, often atelectatic tympanic cavity The object is to enlarge the tympanic cavity preserving the mucosa. The adhesions were removed sparing the mucosa as far as at all possible. Cysts and cholesterol granulomas were opened and their walls removed. In the round window niche there were often adhesions dividing the niche into several segments filled with mucous secretion. In a few cases the round window was widened with a burr Bony and fibrous septa in the hypotympanon were removed, and thereby it was deepened. Frequently there were closed spaces in the tympanic cavity filled with tenacious mucus which was removed. Any mucosal defects on the promontory were covered with silastic film except when situated over the round window or in the hypotympanon

(3) *Tubal orifice and tube* The tubal orifice was closely inspected In several cases it was blocked, either by a greatly retracted drum cyst formations or adhesions. The transition to the osseous tube could often be inspected.

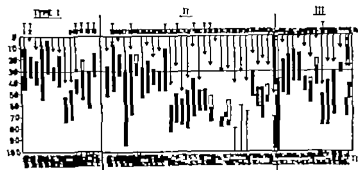


Fig. 1 Results of type I, II and III tympanoplasty. *T* indicate patients who also had tubulation. ■ hearing gain 3–9 months after the operation, □ hearing gain 1–2 months after the operation in cases where the hearing later deteriorated. *TI* stated, at the top before and at the bottom after the operation.

The tube was invariably bougied by a $^1/_2$, $^3/_4$, or 1 mm firm rubber bougie. It could be passed down to the rhinopharynx in all cases but one in which the bougie stopped at the tubal isthmus. In some cases the tube contained a mucous secretion which was sucked up.

(4) The *ossicular chain* was more or less fixed. *The stapes was always surrounded by adhesions*, often tent-shaped with cholesterol cysts in the niche. Cleaning of the stapedial niche was among the most important and in cases of an intact ossicular chain most difficult steps of the operation. To prevent a rapid refixation of the stapes the mucous membrane from the stapedial niche was removed, and the facial as well as promontorial side of the niche was covered with 2 × 3 mm pieces of silastic film. The malleus was usually greatly retracted, adhering at the umbo to the promontory and immobile. In the event of pronounced fixation in the epitympanon, the head of the malleus was excised. The incus was excised or mobilized by means of control openings to the attic or to the aditus. The methods of the ossiculoplasties, all of which had the purpose of enlarging the tympanic cavity will be mentioned in the analysis of the results (Fig. 1). The entire tympanic cavity was filled with penicillin-moistened gelfoam.

(5) *Epitympanon and antrum*. In 20 cases control openings to the attic, aditus, or antrum were made, in most cases through Wullstein's or Heermann's incision. Fibrous adhesions around the ossicles were removed, and the passage from the epitympanon to the tubal orifice was re-established. In a few cases small pieces of silastic film were inserted between the superior epitympanic wall and the ossicles. The mucosa was preserved, and any mucus was sucked out. As all the ears were dry mastoidectomy was not carried out.

The dressing was changed for the first time 3 weeks after the operation. Insufflation of air into the middle ear with Valsalva's manoeuvre was done for the first time at the end of the first week. If after the operation the Eustachian tube was passable in the Valsalva manoeuvre, the patient was instructed to continue with this manoeuvre once or twice daily for several months. If it was not patent, *insufflation of air by catheter* was performed once or twice weekly until Valsalva's manoeuvre could be carried out. The tube was left in the ear for as long as possible. In several cases, however the tube was expelled 1–4 months after the operation.

MATERIAL AND RESULTS

The material comprises 56 patients, 2 of whom (*Fig. 1 cases 34 and 56*) had tympanoplasty twice. 11 of the patients were from 5–20 years of age, 13 were 21–40 years, 25 were 41–60 years, and 7 were over 60. All the ears were dry at the time of operation. The pathological changes of adhesive otitis have been described in detail by Ojala (1953) and Sil-

rala (1964 a). The present material comprises only patients in the adhesive and terminal stages, not in the subacute, initial stage which is generally called secretory or serous otitis.

The analysis of the results is based upon the condition 3–9 months, in most cases 4–6 months, after the operation. Postoperative hearing is taken to mean that in the last, not in the best tone audiogram in the frequency range 500–2 000 cps, and in the last speech audiometry given as the TI (threshold of intelligibility)

As the main principle of the operation was removal of the diseased parts of the drum, the majority of patients got a perforation which was closed with fascia. Recurrence of the perforation occurred in only one case af ter the operation. Perforations following tubulations also closed spontaneously On inspec tion in Siegle s otoscope after the operation the drum was found to have good mobility in 27 cases and it was not retracted. In 16 cases it was slightly retracted, but movable in 7 it was retracted with little mobility and in 6 es it was immobile.

n 13 cases the ossicular chain was preserved tact, and type I tympanoplasty (Fig. 1) was successfully performed. Type II tympanoplasty was done in 30 cases, with interposition of the incus in 25 (cases 14–38) with interposition of polyethylene between the long crus and the stapes in 2 (cases 39–40) of a piece of bone in 2 (cases 41–42) and with transposition of the handle of the malleus in one (case 43). Type III tympanoplasty was done in 13 cases, in 6 (cases 44–51) with interposition of the incus between the footplate and the malleus or the drum and in 2 cases (cases 50–51) with the incus as a homograft. In 4 cases (cases 52–55) the stapes was so firmly fixed that stapedectomy had to be performed Schuknecht s prosthesis was placed either on a defective long crus of the incus or on the handle of the malleus. In one case (case 56) a steel prosthesis was used and in the other a piece of bone between the footplate and the handle of the malleus.

Table I. *Mean pre- and postoperative hearing hearing gain, and air–bone gap in the frequency range 500–2 000 CPS and mean TI for entire material and after various types of tympanoplasty*

	Tympanoplasty			Entire material
	I	II	III	
Mean preoperative hearing	49.2	62.6	58.4	58.5
Mean postoperative hearing	24.0	41.8	30.6	35.0
Mean hearing gain in dB	25.2	20.8	27.8	23.5
Mean preoperative air–bone gap	34.3	40.3	38.5	38.9
Mean postoperative air–bone gap	8.7	20.0	10.8	15.6
Mean preoperative TI	44.3	54.6	51.2	53.0
Mean postoperative TI	21.5	37.0	28.1	31 4
Mean hearing gain in dB of TI	22.8	27 6	23 1	1 6

Calculation of the mean pre and post operative hearing for the frequency range 500–2 000 cps and of the mean TI showed (Table I) that the hearing gain was most marked, 27 8 dB after type III tympanoplasty least marked, 20 8 dB after type II.

The favourable result after type III must be due to there being least difficulty in cleaning the footplate and removing peristapedial adhesions in this type of tympanoplasty

The mean preoperative hearing had been rather poor (58.5 dB), and a number of the patients had severe perceptive hearing loss (Fig. 1) Although the good mean hearing gain in the entire series was 23.5 dB the post operative mean hearing in the entire series was 35 dB (Table I) The mean air–bone gap was reduced from 39 to 15 6 dB.

The usual methods of assessment (Table II) show that no patient had social hearing prior to the operation, but this was attained by 52 after the operation The air–bone gap after the operation had closed within 0–15 dB in 61 % of the cases, while prior to the operation it had exceeded 30 dB in 84 A TI of 30 dB or better was obtained in 55 A hearing gain exceeding 20 dB was obtained in

57% and of more than 10 dB in 86%. Exacerbation was not found in any case.

In other words, the operation was a success in the 29 cases that obtained social hearing in the 10 patients who did not obtain social hearing but in whom the air–bone gap was closed within 0–15 dB, and in the 4 patients whose hearing improved by more than 20 dB although they did not attain social hearing and the air–bone gap did not close within 0–15 dB. Another case must be considered successful, as the postoperative TI was 25 dB, although social hearing was not attained. In all, then, the tympanoplasty proved successful in 44 patients (80%). Among the remaining 12 patients 5 obtained a hearing gain of 11–20 dB.

Tubulation was carried out in 20 cases, as a rule antero-superiorly more rarely antero-inferiorly in the drum remnant. In some cases tubulation had been planned, but the drum remnant was too small, as there must be some distance from the tube to the edge of the graft.

The results (Table III) show that in the cases tubulated at the time of the tympanoplasty the mean postoperative hearing was considerably better the hearing gain greater and the air–bone gap less than in the non-tubulated group. In most of the cases the tube functioned for more than 3 months, in a few for more than 6 months.

The function of the Eustachian tube was reduced in the great majority of cases. As the drum was highly abnormal and there was no or only a very small air space behind it, measurements of tubal function by tympanometry (Terkildsen, 1962) showed a negative middle ear pressure or more often, a blind curve. Tubal passage was assessed by Valsalva's manoeuvre in which the indicator was auscultation in the auditory meatus, otoscopy with or without Siegle's otoscope, and in most cases inspection of the drum by operating microscope. By the latter method changes in the position of the drum could be seen even when the tympanic cavity was very small, for instance in cases where only the tubal orifice was air-filled. Before and after the operation the tube was passable in Valsalva's manoeuvre in 22 cases (Table IV). The most favourable results were obtained in this group, and 86% of the cases were successful. In 19 cases the tube was not passable in the Valsalva manoeuvre prior to the operation, while it was so afterwards. However the tube was passable in Politzer's test or to insufflation of air through a catheter. Within this group the named procedures around the tubal orifice have presumably been successful in several cases, while some patients have perhaps gradually attained more proficiency in the Valsalva manoeuvre. The results in this group too were good, the tympanoplasty being successful in 84% of the cases. However the mean

Table II. *Results assessed by various criteria*

Criterion	Before operation Number		After operation Number	
1. Social hearing (0–30 dB)	0	0	29	52
2. Air–bone gap:				
0–15 dB	0	0	34	61
16–30 dB	9	16	17	31
Exceeding 30 dB	47	84	5	8
3. TI 30 dB and better	3	5	31	55
4. Hearing gain exceeding 30 dB			14	25
exceeding 20 dB			32	57
exceeding 10 dB			48	86
0–10 dB			5	9
No hearing gain			3	5
Aggravation			0	0

Table III. *Results of tympanoplasty with and without tubulation*

	No. of cases	Mean postop. hearing	Mean hearing gain	Mean postop. air–bone gap	Success No.	Success %
With tubulation	20	28.0	27.7	12.5	18	90
Without tubulation	36	39.1	23.8	17.4	26	74

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most critical period immediately after the operation. When the graft has healed and mucosal defects have become epithelialized, there is less risk of re fixation even though the tube has gradually become plugged or has been expelled.

Bougienage of the Eustachian tube has possibly improved the stenosis in some cases. At least, it has not made it worse. In my studies of the mucous glands of the Eustachian tube (Tos, 1970) I have been able to bougie the tube in the course of dissection even in foetuses aged 11 weeks without damaging the tube. Bougienage of the Eustachian tube has afforded objective information about the degree and site of the tubal stenosis and thereby also about the prognosis of the tympanoplasty

The present results go to show that the Valsalva manoeuvre before and after the operation is a fairly reliable indicator of the prognosis of tympanoplasty

Another major problem is continued production of mucus in the middle ear In secretory otitis there are pathological mucous glands in the middle-ear mucosa and an increased number of goblet cells producing the tenacious mucous secretion. In a patient with mild secretory otitis (Bak-Pedersen & Tos, 1971) we found 500 mucous glands in the middle-ear mucosa. Their density ranged from 19 to 1 gland/mm² in the various parts of the middle ear The glands start forming before the patient has developed any symptoms of secretory otitis, they produce mucus through the entire period of secretion, but gradually degenerate, and the production of mucus decreases (Tos & Bak Pedersen 1971).

Since chronic adhesive otitis is a terminal stage of secretory otitis, the mucosa will contain the same number of glands as in secretory otitis, but the great majority—especially in the terminal stage of adhesive otitis—are degenerated and inactive However it was observed in the present material that in some cases the mucosa as a whole was still typically secretory with accumulation of mucus in the middle ear In some other cases mucus was found in delimited areas, i.e. in the epitympanon, in the round window niche, or in closed spaces in the hypotympanon. Accordingly in quantitative studies of glands from mucosal biopsies and removed adhesions in these patients, we have found a large number of mucous glands, several of which were still active and producing mucus. In other cases, with no mucus, we found almost exclusively degenerated glands. Thus, in some cases of adhesive otitus the mucous membrane may go on producing mucus which accumulates in the middle ear and which will later despite a successful tympanoplasty require renewed tubulations and suction of the secretion.

ZUSAMMENFASSUNG

Die Resultate der Tympanoplastik bei 56 Patienten mit chronisch adhäsiver Otitis sind präsentiert. Das Prinzip der Operationsmethoden war eine radikale Entfernung der pathologischen Teile des Trommelfells, Inspektion und Öffnung des Tubaostiums mit Bougienierung des Eustachischen Rohres, Mobilisation der Gehörknöchelchen, Anwendung von solchen Ossikuloplastiken, die das Mittelohr vergrößern, Einlage des Silastic Films in die Stapesnische und Tubulation. Die durchschnittliche Verbesserung des Gehörs in der Frequenzgruppe 500–2 000 Hz, 3–9 Monate nach der Operation war 23,5 dB, das postoperative Gehör 35 dB und Mittelohrkomponente 15 [illegible] In 80% der Fälle wurde die Tympanoplastik erfolgreich angewandt und 86% der Fälle bekam eine [illegible]verbesserung, die größer war als 10 dB. Die besten Resultate wurden bei solchen Fällen gefunden, [illegible] gleichzeitig mit der Tympanoplastik tubuliert war [illegible] Die Resultate wurden in Verbindung mit der Tubenfunktion bei Valsalva's Versuch vor und nach der Operation analysiert. Die besten Resultate wurden bei solchen Fällen gefunden, wo das Eustachische Rohr vor und nach der Operation durchgängig [illegible] die schlechtesten dort, wo es weder vor noch nach der Operation durchgängig war

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Acta Otolaryng 73 61–63 1972

STUDIES OF LDH ACTIVITY CONCERNING MALIGNANT TRANSFORMATION FROM CHRONIC SINUSITIS

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(Received June 1 1971)

Abstract The LDH activity of maxillary cancer tissues was considerably higher than in the other tissues including the surrounding tissues, irradiated cancer tissues, and the mucosa of the maxillary sinus in chronic sinusitis. From the standpoint of classified LDH isozyme patterns, the maxillary cancer tissues were characterized by predominant fractions of LDH_4 and LDH[illegible] in contrast to the chronic sinusitis tissues in which LDH[illegible] prevailed. We designated the type of the former "Cancer type" and the latter "Inflammation type" and proposed designation of ten types of the LDH isoenzyme pattern (from 1 to 10). The enzymologic studies on the basis of the LDH activity and isozyme aspect classified that the histologically proliferative type in chronic sinusitis resembles the cancer type.

Patients with maxillary cancer treated at Osaka University Hospital over the past 11 years numbered 429 of whom those complicated with sinusitis accounted for 344 patients, or 80.3% The mass examinations in schools and companies, as well as the data published in 1965 both of which were published by the Japanese Ministry of Health and Welfare, estimated the incidence of chronic sinusitis to be about 10% Furthermore, the estimated mortality from maxillary cancer is 1 000 annually throughout Japan. These data indicate that patients having sinusitis are exposed to the risk of contracting malignant transformation at a probability of 1% throughout his life, in contrast to those persons without chronic sinusitis, who have a far lower coefficient of 0.03%

It is known that the level of lactic acid generally increases in blood passing through tumors of experimental or human origin This has been ascribed to the increased glycolysis and/or decreased oxidation of lactic acid. Meister investigated the LDH activity in 20 normal human tissues and 19 different tumors in mice and rats. It has been made clear that the LDH activities of the tumors are equal to or higher than, those of corresponding normal tissues. In the present investigation, the LDH activity was studied enzymologically and for the isozyme patterns, which are contained in tissues of the paranasal mucosa of maxillary cancer as well as of chronic sinusitis.

Tissue specimens removed at operation, either intact or thawed after being stored under refrigeration, were weighed, diced, and homogenized in an all-glass Potter Elvehjem *motor-driven homogenizer with the aid of a* cold Veronal buffer solution so that each ml of the finished homogenate contained 100 mg of the specimen. The homogenate was centrifuged at 3 000 *g* at 4 C for 1 hour and the supernatant fluid was immediately frozen for later assay The enzyme activity of this specimen was expressed in terms of mg nitrogen.

We studied the LDH activity in the paranasal mucosa of patients with various diseases. 26 patients with maxillary cancer whose cancer tissues and the surrounding areas were compared 69 patients with chronic sinusitis,

Table I. *LDH activity in the tissues*

	Number of cases	Reliable limits of average (U/mgN)
Maxillary cancer		
Untreated tumor	26	2 910 ± 660
Tissue surrounding the tumor	16	1 290 ± 650
After irradiation	5	1 540 ± 1270
Chronic sinusitis		
Histological type of paranasal mucosa		
Proliferative	24	1 530 ± 540
Inflammatory	18	1 560 ± 430
Edematous	27	1 310 ± 310
Subtotal	69	1 440 ± 240
Nasal mucosa	25	1 420 ± 580
Nasal polyp	10	960 ± 850
Total	104	1 350 ± 200
Ciliated epithelium		
Normal	34	1 070 ± 290
Partially defective	27	1 510 ± 420
Wholly defective	43	1 550 ± 380
Squamous epithelial metaplasia	9	1 630 ± 1110
Glandular proliferation	50	1 490 ± 310
Hemorrhage	26	1 280 ± 320

10 patients with nasal polyps, and 5 post-ir ... patients with maxillary cancer. In ...nts with maxillary cancer receiving no ...ent the LDH activity was significantly ...gher as Table I shows.

The paranasal mucosa in chronic sinusitis was divided into three different groups, histologically into proliferative, inflammatory and edematous types and according to the property of the epithelium, with normal cilia and with defect either partial or whole. The LDH activity of the mucosa was also calculated on various specimens, including one from squamous epithelial metaplasia, glandular proliferation, and hemorrhage according to the above-mentioned classification (Table I).

The LDH can be separated electrophoretically into five different isozymes, viz. from LDH_1 to LDH_5. The enzymes, obtained from patients with maxillary cancer as well as from patients with chronic sinusitis, were characterized by their higher activities of fractions of LDH_3, LDH_4 and LDH_5 than those of LDH_1 and LDH_2. Furthermore, it became clear that, LDH_4 and LDH_5 predominated over LDH_3 in maxillary cancer and that LDH_3 was the highest in chronic sinusitis. We designated the former type "Cancer type" and the latter "Inflammation type". We propose a designation of ten types of the LDH isozyme pattern covering these two types (Fig. 1). Untreated maxillary cancer differed slightly from recurred one in that the former prevails in the LDH isozyme patterns from 1 to 5 and the latter in those from 1 to 8. After irradiation the LDH activity from maxillary cancer was halved. Of the 9 patients treated by irradiation, 4 fell in types from 1 to 5, and the remaining 5 from 6 to 10.

The LDH activity of the surrounding tissue of this cancer was about one-half that of the cancer tissue, with types 1 to 5 in 5 patients and types 6 to 10 in 11 patients. It is of great interest that as many as 5 of the 16 patients with maxillary cancer showed in the surrounding non-cancer tissues the LDH activity pattern of types 1 to 5, which were rarely

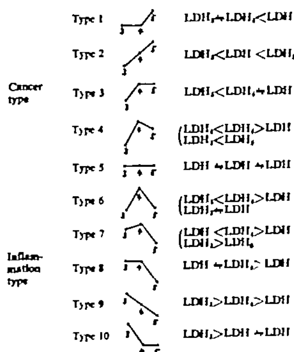


Fig. 1. Types classified by LDH isozyme pattern.

Table II. *Ranging of LDH isozyme pattern (number of cases)*

	Type of LDH isozyme pattern										
	1	2	3	4	5	6	7	8	9	10	Total
Maxillary cancer											
Untreated tumor	1	3	5		7			10			26
Tumor recurred	1		2		2	2	1	2			10
After irradiation			1		3	2		2		1	9
Tissue surrounding the tumor			1	3	1	2	2	5	2		16
Chronic sinusitis											
Histological type of paranasal mucosa											
Proliferative						5	10	7	3		25
Inflammatory						2	8	9			19
Edematous						2	9	17	3	1	32
Subtotal						9	27	33	6	1	76
Nasal mucosa		1		1	1	8	3	11	1	1	27
Nasal polyp			1	1		3	2	2		1	10
Total		1	1	2	1	20	32	46	7	3	113
Ciliated epithelium											
Normal			1			5	12	18	3		39
Partially defective		1		1	1	6	5	11	1	2	28
Wholly defective				1		9	15	17	3	1	46
Squamous epithelial metaplasia		1		1		3	1	2		1	9
Glandular proliferation				1		11	14	21	4		51
Hemorrhage						3	15	7	1	2	28

found in any tissue of the mucous membrane of chronic sinusitis.

It has been elucidated that the histologically proliferative type of chronic sinusitis shows a tendency to resemble the cancer type in its isozyme pattern. The LDH activity of the mucosas with squamous epithelial metaplasia also shows a similar tendency (Table II).

Our studies performed on the bases of LDH activity and its pattern may indicate the significance of treating chronic sinusitis from the viewpoint of preventing maxillary cancer.

ZUSAMMENFASSUNG

Die Enzymaktivitäten der LDH in der chronisch entzündeten Kieferhöhlenschleimhaut und im Tumorgewebe bei Oberkieferkrebs wurden gemessen. Die Aktivität der LDH im Krebsgewebe zeigte einen hohen Wert und der Isozymspiegel zeigt, daß die Konzentration der LDH₅ anstieg. Die LDH-Aktivität im chronisch entzündeten Gewebe und im Nachbargewebe des Tumors ergaben dagegen einen niedrigeren Wert. Diese niedrige LDH-Aktivität ergab sich auch im durch Behandlung histologisch gebesserten Tumorgewebe.

Es wird aus diesen Ergebnissen des Isozymspiegels darauf hingewiesen, daß eine mögliche Beziehung zwischen der Aktivität der LDH und der malignen Entartung des Gewebes bestehen kann.

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Acta Otolaryng 73 64–67 1972

THE EFFECT OF TRIAMCINOLONE ACETONIDE ON ALLERGIC AND VASOMOTOR RHINITIS

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(Received April 13 1971)

Abstract. A double-blind technique was used to evaluate the effect of Triamcinolone Acetonide on ninety-seven severe cases of well-defined allergic and vasomotor rhinitis. A placebo or 40 mg of the long-acting steroid was injected intramuscularly The evaluation of the effect was made after 10 days. Patients who were not improved then received another injection, always of Triamcinolone Acetonide. The steroid had a statistically significant effect on the nasal symptoms both in patients with allergic and vasomotor rhinitis, particularly apparent in the former The need for great care in prescribing such a potent drug, particularly in the more or less continuously severe vasomotor cases, is emphasized.

Allergic patients with symptoms of severe rhinitis are common in otolaryngological prac Some of these patients may be extremely disabled during the pollen season. Others have less severe but constant non-infectious nasal symptoms throughout the year causing many problems not only for the patient but also for the doctor

Corticosteroids have now been in use for two decades in the treatment of allergic disorders. Modern derivatives of cortisone and cortisol are introduced continuously and their effects (and side-effects) are well recognized (Bondy 1962 Brown 1962 Cope 1965 Lindholm 1967) However there is a great lack of controlled studies on the effect of corticosteroids on non-infectious rhinitis. The purpose of this study was to evaluate the effect of a new long-acting corticosteroid (Triamcinolone Acetonide, Kenacort® T susp. for injection 40 mg/ml, Squibb) on a number of well defined patients with allergic and vasomotor rhinitis.

MATERIAL AND DEFINITIONS

The study consists of 97 selected patients with severe symptoms of non-infectious rhinitis. Thirty-eight patients suffered from *allergic rhinitis* and 59 from *vasomotor rhinitis.*

Allergic rhinitis is here defined as: Patients with a distinct period with severe symptoms during the summer being free from symptoms during the rest of the year The allergens were in all cases different kinds of grass pollens, previously confirmed both by intradermal and provocation-tests. All patients with allergic rhinitis had suffered from severe symptoms during at least three earlier periods and were all well-known to one of us (B Lindholm). Adequate examinations as to the allergological aspects were made prior to the study

Vasomotor rhinitis is here defined as. All other kinds of non-infectious rhinitis, elicited by other than allergic factors. The patients with vasomotor rhinitis had suffered from severe symptoms more or less continuously for at least 1 year The patients with vasomotor rhinitis were also thoroughly investigated and were selected for the present investigation by one of the authors (A Axelsson)

Table I

	Allergic rhinitis	Vasomotor rhinitis
Number of patients	38	59
Men	16	24
Women	22	35
Mean age	28 (18–51)	38 (22–76)
History (Chief complaints)		
Nasal obstruction	38	50
Watery nasal discharge	34	42
Sneezing	26	30
Nasal itching	15	28
Impaired smell	12	26
Conjunctival itching	12	28

The material and the patients chief complaints are presented in Table I. None of the 97 patients received any other symptomatic therapy during the observation period.

METHODS

The study was designed as a double-blind investigation. At random, the patients received an intramuscular injection of either 40 mg of Triamcinolone Acetonide or of a placebo The therapeutic effect was evaluated 10 days later Unimproved or deteriorated patients then received another injection, this time always consisting of Triamcinolone Acetonide. All patients were re-evaluated after further 10 days. The patients with allergic rhinitis were studied during two seasons (1969 and 1970) in 1970 without the placebo-technique.

The patients with vasomotor rhinitis were subjected to radiological examination of the sinuses prior to the study All initial radiological sinus changes were reviewed after 10 days.

Further the sneezing frequency was analysed in those patients with vasomotor rhinitis, giving a history of more than 10 sneezes per day just prior to the study Only cases where the frequency was reduced to more than half of the initial frequency were considered to be improved.

RESULTS

The patients' subjective evaluation of the clinical course during the observation time is given in Table II.

Patients with allergic rhinitis

After 10 days, 16 out of 17 patients were restored or improved after Triamcinolone Acetonide treatment. The unimproved patient was

Table II *Subjective evaluation of the clinical course*

	No	After 10 days		After further 10 days	
		Restored or improved	Un-improved*	Restored or improved	Un-improved
Allergic rhinitis					
1969					
Triamcinolone acetonide	17	16	1	0	1
Placebo	21	2	19	16	3
1970					
Triamcinolone acetonide	35	31	4		
Vasomotor rhinitis					
Triamcinolone acetonide	34	27	7	3	4
Placebo	25	11	14	11	3

* Patients who were unimproved after 10 days received another injection always containing 40 mg Triamcinolone acetonide. The clinical course in these cases was again analysed after further 10 days.

not improved by another Triamcinolone Acetonide injection. Of 21 patients given placebo, 19 were unimproved. Of these 19 subjects, 16 were improved by the subsequent injection of Triamcinolone Acetonide and evaluated after another 10 days.

In 1970 35 of the 38 patients with allergic rhinitis received another Triamcinolone Acetonide injection, when symptoms reappeared. Thirty-one patients were improved.

Patients with vasomotor rhinitis

Twenty-seven of 34 patients receiving Triamcinolone Acetonide were subjectively improved after 10 days. Another 3 patients were improved by a second Triamcinolone Acetonide injection. Of the 25 patients given placebo 11 regarded themselves improved. The remaining 14 cases received a second injection (containing Triamcinolone Acetonide) and of these, 11 were improved after further 10 days of observation.

The present investigation thus demonstrated that Triamcinolone Acetonide has a statistically significant effect on both allergic and vasomotor rhinitis.

Radiological sinus changes were found in 28 patients with vasomotor rhinitis (48%). The sinus changes were unchanged after 10 days in more than half of the patients treated both by Triamcinolone Acetonide and placebo. No statistical difference in the effect of Triamcinolone Acetonide or placebo on the sinus changes could be demonstrated.

The effect of Triamcinolone Acetonide on the sneezing frequency in patients with vasomotor rhinitis, is shown in Table III. A statistically significant difference was found between the patients receiving Triamcinolone Acetonide when compared with those receiving placebo. The sneezing frequency was reduced after Triamcinolone Acetonide.

No serious side effects were noted. Two patients treated with Triamcinolone Acetonide complained of menstrual disturbances and two others of local tenderness after the injection.

Table III *Sneezing frequency in patients with vasomotor rhinitis before and after treatment*

	Number of patients		
	Before treatment	After 10 days	
	Sneezing[a]	Improved[b]	Unimproved
Triamcinolone acetonide	22	18	4
Placebo	16	5	11

[a] Only patients sneezing more than 10 times a day are analysed.
[b] Sneezing frequency reduced by more than 50 %.

DISCUSSION

In a minority of patients suffering from allergic rhinitis and vasomotor rhinitis, conventional therapy such as elimination of allergens, hyposensitization, antihistaminics etc. ceases to have sufficient effect. These patients are severely disabled and often invalidated socially. In our opinion, symptomatic treatment with corticosteroids should be reserved for this category of patients. Thus, all patients selected for the present study belong to this group by definition. In previous reports on the effect of corticosteroids in patients with non-infectious rhinitis (Schwartz & Avram 1963, Riedel 1967, Sherwood et al. 1967, Dols, 1968) the severity and duration of the disease has been rather vaguely described. Moreover the disorders treated have not been defined properly which makes it difficult to compare the results of different investigations. There is urgent need for better clinical methods to evaluate the effects of drugs on both allergic rhinitis and vasomotor rhinitis. This statement holds true especially with regard to effects on the latter disorder. In clinical trials on effects of corticosteroids on non-infectious rhinitis (allergic rhinitis and vasomotor rhinitis) it must be regarded as a necessity to use the double-blind technique. This was done in the present investigation. Further attempts were made to objectively evaluate the clinical course by radiological examinations of sinus changes and

by analysing the sneezing frequency before and after treatment in the patients with vasomotor rhinitis. The radiological examination, however demonstrated that sinus changes in general were little influenced by Triamcinolone Acetonide. Contrarily the analysis of the effect on the sneezing frequency clearly demonstrated the effect of Triamcinolone Acetonide in patients with vasomotor rhinitis.

It seems to be beyond doubt that Triamcinolone Acetonide was effective in practically all the patients with allergic rhinitis. A significant effect was also demonstrated on the patients with vasomotor rhinitis, even if the results were not as impressive as in the cases with allergic rhinitis. Actually these findings are not surprising with regard to our definition of the disorder and the knowledge of the complex aetiology of vasomotor rhinitis.

The observation time of 20 days does not permit any conclusions about the exact duration of the effect of 40 mg Triamcinolone Acetonide given intramuscularly. It may however be stated that those patients who were improved after Triamcinolone Acetonide treatment all noticed an effect lasting more than 10 days. It seems likely that the effect was more prolonged in many of the patients.

Triamcinolone Acetonide thus had a prompt and longstanding effect in patients with allergic rhinitis and vasomotor rhinitis. However it cannot be recommended for routine purposes, particularly not in the patients with vasomotor rhinitis. They will demand this potent remedy at every recurrence when the risk of side effects will increase considerably. Even in patients with allergic rhinitis with a limited and well defined period of symptoms, the drug—as any corticosteroid treatment—has to be reserved for the most severe cases, where all conventional therapy has failed.

ZUSAMMENFASSUNG

Eine Doppel-Blind-Technik wurde angewendet, um die Wirkung von Triamcinolon Acetonid ([illegible] Squibb) bei 97 Fällen von schwerer [illegible] allergischer oder vasomotorischer Rhinitis zu untersuchen. Ein Placebo oder 40 mg des [illegible] Steroids wurden intramuskulär injiziert. Die Auswertung des Effektes erfolgte nach zehn Tagen. Patienten, bei denen keine Besserung eingetreten war erhielten dann eine weitere Injektion, die immer aus Triamcinolon Acetonid bestand. Der Effekt des Steroides auf die Nasensymptome war statistisch signifikant besser als der des Placebo, dieses sowohl bei vasomotorischer wie auch besonders bei allergischer Rhinitis. Es wird betont dass es notwendig ist [illegible] der Verschreibung eines so potenten Mittels vorsichtig zu sein, besonders bei den ständig mehr oder weniger invalidisierten Fällen mit vasomotorischer Genese.

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Acta Otolaryng 73 68–74 1972

MUCO-EPIDERMOID CARCINOMAS OF THE SALIVARY GLANDS

With Special Reference to the Possible Existence of a Benign Variety

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(Received June 1 1971)

Abstract It has been questioned whether there is a benign variety of muco-epidermoid carcinoma (M.E.C.) with histologically highly differentiated structures. To find out whether such a definitely benign variety really exists a histological investigation was made of [illegible]3 muco-epidermoid carcinomas with a malignant course. These 23 cases were selected from 1[illegible]4 which, after histological reclassification of [illegible]867 parotid, submandibular and palate tumours, were classified as M.E.C In 7 of the 23 cases the histological study revealed highly or moderately differentiated structures, and in 3 cases the primary tumour as well as the lymph node metastases were undoubtedly very high differentiated. These [illegible]logically benign structures in cases with a [illegible]lignant course would contradict the existence of a [illegible] variety of M.E.C., and consequently tumours with highly differentiated structures should be denoted as carcinomas in preference to the sometimes suggested term of muco-epidermoid tumour

Muco-epidermoid carcinoma (M.E.C.) of the salivary glands is nowadays recognized as a distinctive type of tumour (Stewart et al. 1945 Linell, 1948 Eneroth 1964). Histologically the tumours may appear to be more or less differentiated. Most authors have distinguished between a highly differentiated ("low grade malignant") or poorly differentiated ("high grade malignant") type Since the borderline between highly and poorly differentiated tumours may be diffuse Foote & Frazell (1954) also suggested a third, intermediate group

In reports of M.E.C in the submandibular gland (Eneroth et al., 1967) and in the palate (Eneroth et al., 1970), highly differentiated tumours were distinguished from poorly differentiated. The correlation study between the histological and clinical features revealed that no patient with a highly differentiated M.E.C. died of the tumour disease or had metastases. In a corresponding study of M.E.C. developing in the parotid gland (Jakobsson et al., 1968) 2 patients with highly differentiated tumours were reported to have died of the tumour disease.

It is well known also from reports other than the above-mentioned that highly differentiated M.E.C. has a good prognosis (e.g. Woolner et al., 1954 Bhaskar & Bernier 1962) and it has also been questioned whether these tumours should be considered benign and hence be denoted as muco-epidermoid tumours rather than carcinomas.

The aim of the present study was to ascertain on morphological grounds whether a definitely benign variety of muco-epidermoid tumour can be delineated. For this purpose a histological investigation was made of all available M.E.C. with documented malignant character either through the presence of histologically verified metastases or through the death of the patients in the tumour disease a[illegible] morphologically benign structures of M.E.C

Table I *Clinical findings in 23 cases of muco-epidermoid carcinoma with a malignant course of the disease*

Location of primary tumour	No. of cases Total	Men	Average age at onset	Local extension of tumour outside the capsule n	Local recurrences n	Histological verified metastases n	Died of tumour disease n
Parotid gland	15	9	52	10	11	9*	15
Submandib. gland	2	2	45	2	2	2	2
Palate	6	5	70	6	2	1	5
All cases	23	16	56	18	15	13	22

Besides in regional lymph nodes, 2 patients had skin metastases simultaneously

(highly differentiated) in cases with a malignant course would contradict the existence of a benign type of muco-epidermoid tumour

MATERIAL

In a survey of 2 867 parotid, submandibular and palate tumours in patients treated at Radiumhemmet and the Department of Otolaryngology Karolinska Sjukhuset, during the period 1919–1969 a histological reclassification revealed 2 513 salivary gland tumours. The present investigation is based upon a histological and clinical study of the 124 tumours which were classified as M.E.C. (about 5%). The relative incidence of M.E.C. was found to be highest in the palate—16% (30 of 185 cases), whereas in the parotid and submandibular gland the incidence was about 4% (parotid gland 88 of 2 158 submandibular gland 6 of 170) Since none of the 124 patients were lost from the follow-up study and the tumour material in all cases was available at the Department of Tumour Pathology it was possible to extract for this study all cases of tumours with documented malignant character either through histologically verified metastases or through the death of the patients in the tumour disease. A total of 23 of such tumour cases with malignant course were found.

RESULTS

The clinical findings in the 23 cases of M.E.C. with clinically manifested malignant character are presented in Table I It is evident from the table that these types of tumour are a little more common in men, especially in the submandibular gland and in the palate. The average age at onset of the disease was considerably higher for tumours originating in the palate than in the major salivary glands. The tumours of the submandibular gland and the palate region had a greater tendency to local extension with growth beyond the capsule compared with the parotid tumours.

Histologically verified metastases were found in about half of the cases. All these *metastases were found in regional lymph* nodes but, in two of the 9 primary parotid tumours, metastases to the subcutaneous tissue in the neck region were found as well. Only 1 patient is alive at the end of the follow-up period. The observation period in this case (a solid tumour in the hard palate with lymph node metastases to the right side of the neck) is, however only 15 months.

The histological survey of the tumours in the 23 cases revealed their character of M E.C. Thus epidermoid cells and mucus-secreting goblet cells were found in varying proportions in all of the primary tumours. In all tumours so-called intermediate cells were also present.

In the histological classification, obviously highly differentiated tumours were distinguished from poorly differentiated, mainly on the basis of generally accepted morphological criteria such as degree of cellular maturation and polymorphism as well as cellular

Fig. 1. Lymph node metastases of highly differentiated M.E.C. Photomicrograph, 160. Woman, 36 years, with primary parotid tumour, died of the cancer after 5 years.



Fig. 2. Lymph node metastases of highly differentiated M.E.C. Photomicrograph, 160. Man, 62 years, with primary tumour in the soft palate, alive 15 months after onset.

Fig 3 Subcutaneous metastases of highly differentiated M.E.C. Photomicrograph, 160. Woman, 59 years, with primary parotid tumour died of the cancer after 3 years.

Fig 4 Subcutaneous metastases of m[illegible] differentiated M E C Photomicrograph, [illegible] 29 years, with primary parotid tumour died of after 2 years.

Fig. 5. Lymph node metastases of moderately differentiated M.E.C. Photomicrograph, × 160. Woman, 51 years, with primary parotid tumour, died of the cancer after _ years.

Fig. 6. Cutaneous metastases of poorly differentiated M.E.C. Photomicrograph, × 160. Woman, 47 years, with primary parotid tumour, died of the cancer within 1 year.

Table II *Type of tumour in 23 cases of muco-epidermoid carcinoma with a malignant course of the disease*

Location of primary tumour	No. of cases	Highly differ ("low grade mal. tumour")	Moderately differ ("intermediate type")	Poorly differ ("high grade mal. tumour")
Parotid gland	15	2	3	10
Submandib. gland	2	—	1	1
Palate	6	1	—	5
All cases	23	3	4	16

composition. A relatively large number of mucus-secreting goblet cells were thus encountered, indicating a high degree of differ entiation of the tumour structure A few borderline cases were found which were classified as moderately differentiated ("inter mediate types") Metastatic lesions were analysed in 13 of the 23 cases. No obvious dif ferences was found between the tumour struc ture in the metastases and the primary tumours. It may be noted, however that in a few cases radiation changes in metastatic lymph nodes were observed secondary to the preoperative irradiations, though these altera tions were of minor magnitude and did not influence the histological evaluation of the tumour structures.

In Figs. 1–6 some examples are shown of the tumour structures in metastases from M.E.C with different degrees of cellular dif ferentiation. From the figures it is obvious that mucus-secreting goblet cells are present within the metastases from tumours classified as highly and moderately differentiated. The illustrated cases of metastasizing, highly dif ferentiated tumours exhibited only little or moderate cellular polymorphism, which is con sistent with what generally would be accepted as a low grade malignant tumour structure.

The results from the histological survey of the 23 cases with malignant M.E.C. in the present material are given in Table II. Three cases were registered as highly differentiated, 2 in the parotid gland and 1 in the palate The tumour structure in these cases was uniform throughout the tumour tissue and corresponds undoubtedly to the type of M.E.C. which would be diagnosed as highly differ entiated tumours with low grade malignancy Of the 23 cases 4 were denoted as moderately differentiated (Figs. 4 5) It may be questioned whether tumours of this type would not be diagnosed by numerous pathologists as highly differentiated tumours. Thus the in vestigation has revealed that, of the 23 cases of M.E.C. with malignant clinical course, no less than 7 (or at least 3) deserve the diagnosis low grade malignant muco-epidermoid car cinoma from the histological point of view

DISCUSSION

It is well established that M.E.C. of the highly differentiated type has a good prognosis, with low frequencies of local recurrences and meta stases and high survival rates (e.g. Eneroth et al., 1967 1970) The true malignant char acter of highly differentiated M.E.C. has been questioned (Stewart et al., 1945 Marcial-Rojas & Sommers, 1954).

In the present investigation the clinical findings have served as the basis for the study From a material of 124 cases of M.E.C. those have been selected in which the follow-up study has revealed a malignant course of the disease. A total of 23 cases were found to fulfil these criteria either through the presence of histologically verified metastases or through the death of the patients in the tumour disease

The results of the investigation show that metastases may occur from M.E.C. which un

doubtedly deserve to be denoted as highly differentiated. It would not be possible on morphological grounds to distinguish between such a type of metastasing, highly differentiated tumours and a histologically hypothetically benign muco-epidermoid tumour The findings do not contradict the low potential malignancy of highly differentiated M E.C. Even if metastases are rare and the survival rates extremely high compared to other malignant salivary gland tumours (Eneroth, 1972, in press) the mere existence of single cases of highly differentiated M E.C. with a malignant course is evidence that such tumours should be regarded as true malignant Consequently we prefer to denote them as muco-epidermoid carcinomas rather than merely as tumours.

ZUSAMMENFASSUNG

Es ist diskutiert worden, ob eine benigne Variante des Mucoepidermoid-Karzinoms mit histologisch hoch differenzierten Strukturen existiert. Um klarzustellen, ob eine solche zweifelsfrei benigne Variante wirklich existiert, wurde eine histologische Untersuchung an 23 Mucoepidermoid-Karzinomen mit malignem Verlauf vorgenommen. Diese 23 Fälle wurden aus 1 4 Fällen ausgewählt, die nach histologischer Reklassifizierung von 867 parotiden, submandibularen und palatalen Tumoren als Mucoepidermoid-Karzinome klassifiziert worden waren. In 7 dieser 23 Fälle wies die histologische Untersuchung hohe oder mässig differenzierte Strukturen auf und in 3 Fällen waren Primärtumor sowie Lymphknotenmetastasen zweifellos sehr hoch differenziert. Diese morphologisch benignen Strukturen in Fällen mit malignem Verlauf widersprechen an und für sich der Existenz einer benignen Variante von Mucoepidermoid-Karzinomen, und Tumoren mit hoch differenzierten Strukturen sollten demnach also als Karzinome und nicht, wie gelegentlich vorgeschlagen, als *Mucoepidermoid-Tumoren* bezeichnet werden.

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ACTA OTO-LARYNGOLOGICA
Vol. 73 No. 2-3

COMPTE RENDU
DE LA RÉUNION SCIENTIFIQUE DU

COLLEGIUM OTO-RHINO-LARYNGOLOGICUM

AMICITIAE SACRUM

ROTTERDAM, LE 25-29 JUILLET 1971

Almqvist & Wiksells

BOKTRYCKERI AKTIEBOLAG

UPPSALA 1972

INTRODUCTION

W H. Struben

Mijnheer de Decaan van de Medische Faculteit Rotterdam,
Members of the Collegium
Ladies of the Collegium,
Honoured guests,
it is my great pleasure to welcome you to the thirty-seventh meeting of the Collegium in Rotterdam.
Mesdames et Messieurs, Membres du Colle gium, je vous souhaite le bienvenue à Rotter dam.
Meine Damen und Herren, ich heisse Ihnen herzlich willkommen in Rotterdam.

It is indeed a great honour that you have come in such large numbers to Holland, the homeland of the Collegium, founded at Groninque in 1926.

I would like to welcome especially one of our members. One who was present and witness of the birth of this organisation. Mon cher ami et le Doyen des Oto-Rhino-Laryngologistes hollandais, le Professeur Eelco Huizinga, qui était présent à la naissance du Collegium en dix neuf cent vingt six et qui était pendant beaucoup d'années comme secrétaire général du Collegium the prancing horse before the carriage who was during so many years the *stimulating force of our organisation.*

I regret to announce that Sir Victor Negus, the Grand Old Man who at his age is still very much alive and will not pass over a single meeting of the Collegium, if he can help it, has written that he is unable to attend this meeting on account of his health but wishes the meeting every success.

Es sind schon zuletzt viele Jahren her dass die Sitzung des Kollegiums in Holland statt gefunden hat. In den 45 Jahren seines Bestehens ist es heute erst das dritte Mal. Obwohl ich mir dessen genau bewusst bin, dass es noch viele Länder gibt, die niemals in der Lage waren als Gastherr oder Gastherrin das Kollegium empfangen zu können, möchte ich doch eine Lanze dafür brechen, alle zehn Jahre jedenfalls einmal zur Heimat des Kol legiums zurückzukehren.

Ich kann Ihnen versichern, dass die holländischen Mitglieder Sie immer gastfreundlich und mit Freude empfangen werden.

Rotterdam, the largest harbour in the world, is proud to be your host. We have had less than a year to prepare this meeting, because only last year in September was it decided that you would accept our invitation. This means that we had little time to solve the many problems connected with the organisation of a meeting· on the other hand this gave us also less than a year to worry instead of two.

We have done our best to make you feel at home and to present you not only a well selected scientific programme by courtesy of the goodwill of our members and guests, but also a pleasant social programme for our ladies and those members who need a little "diver tissement" during or after the working hours of the meeting.

On the cover of the programme you find a picture of the laboratory building of the New Medical Faculty of Rotterdam. This city of *900 000 inhabitants* has started this medical faculty in 1966. The medical history of Rotter dam, however is very much older than that.

Round 1600 Holland had six universities of which Leiden, 1575 was the oldest and six Illustre Schools, one of which was situated in Rotterdam and founded in 1681 An Illustre School was a university based on only one or two faculties. As Rotterdam was situated at one of the busiest rivers in Europe and al ready an important business centre, the ma gistrates of Rotterdam were much more interested in business than in culture they

wanted the most for a minimum cost All professors and lecturers were therefore nominated honorary professors with no or only very little payment. If a professor or lecturer had the misfortune to die, then that was that. The city magistrates decided that no vacancy existed, the salary if any was confiscated and no successor was nominated—a situation that was hardly ideal to build up a normal medical faculty At the end of the 18th century this Illustre School at Rotterdam consisted only of lectures in the following subjects: one in anatomy surgery and obstetrics one in pharmacy and one in natural history and herbs.

A change came when on account of a Royal Decree of 1823 to have more facilities for higher education the magistrates of Rotterdam decided in 1828 to start a new medical school. Several internationally well known lecturers were nominated in the same year The school started in its first year with 108 students. Still there was not sufficient support, nor interest from the government and the city magistrates to develop this school into a real university and after only 38 years of unending struggle for money and recognition of its cul-
value the existence of this medical school
simply ended by another Royal Decree in

After the Second World War for the third time, in 1948 a clinical school was started, this time on the initiative of Leiden and Utrecht universities to help them out with the large number of medical students. All the preclinical training was done in Leiden or Utrecht and for the clinical work the students had a choice between Leiden and Rotterdam or Utrecht and Rotterdam The clinical school was a great success and its work has continued till this month. From the first of September 1971 all will be taken over by our new Medical Faculty Only as recently as 1966 the government decided that Holland needed a seventh full medical faculty and the place to start this faculty should be Rotterdam.

We started building in 1966 and in the same year received 160 first year medical students. Now after 5 years we have a total intake of 800 students. History however has not changed much and getting sufficient money and manpower for completing this medical faculty still has problems. I can assure you that this time however we are determined to finish the job. An important point is that the magistrates of this city now very well realise that no other town of this size anywhere in the world exists without a university

A city of this size needs a cultural influence if it wants to stay ahead in competition and if it wants to attract sufficient people of a certain level of education. There is no town of any importance that can allow itself not to have an institution of higher education. But enough of the medical problems of Rotterdam.

Here in this city on the night of the 27th of October 1469 Erasmus was born, the great humanist, who dedicated his whole life to the education of others. To preach in word, but mostly in poems, letters and books, friendship and freedom, peace and humanity A man who studied all his life and who travelled in the days of the 15th and 16th century all over western Europe to learn from other scientists, wiser men than he was, and to teach others who knew less than he did, but above all to seek friends and make friendship, to seek freedom and make peace

It is no coincidence that our Collegium has chosen Rotterdam as a meeting place for its forty-fifth anniversary The Collegium a scientific organisation founded on friendship, understanding, goodwill and appreciation of others.

It is up to us members and ladies of the Collegium to prove that the words and spirit of Erasmus are still valid and have not changed in five centuries.

May I end by wishing you all a pleasant time and may you leave Rotterdam at the end of this week a much wiser but maybe also a little poorer man, but with many renewed and a lot of new friendships.

I like now to call on you Mr Dean to open the thirty-seventh meeting of the Collegium.

Acta Otolaryng 73 79–86, 1972

MICROHARDNESS TESTING AS A MEANS OF ANALYSING THE MINERALIZATION OF THE OTOSCLEROTIC STAPES

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Abstract. The microhardness of 18 otosclerotic and 9 normal stapedes was tested in order to analyse the degree of mineralization. The analysis was carried out with the Leitz-Durimet on specimens fixed in alcohol and embedded in methacrylates. It was found that the otosclerotic bone is less mineralized than the normal bone The mineral content of the active foci is 10–15 less than that of the normal bone and the quiescent foci are only 1.2–4% less mineralized. The values of microhardness were compared with the data obtained by microradiography

In a previous investigation (Roberto et al., 1972), microradiographic evidence were presented on the distribution of mineral salts in otosclerotic stapedes. The data indicated that the X ray absorption of both active and quiescent foci was constantly lower than that of the normal stapes. Since the differences in the X ray absorption are mainly due to the contents in mineral salts, it was concluded that the mineralization of the otosclerotic bone was lower than that of the normal bone.

When discussing the validity of this conclusion, it is important to consider that the results of the microradiographic technique are strongly influenced by the holes existing in the bone (Jowsey 1964). The presence of numerous large osteocyte lacunae and canaliculi could account for a low X ray density value, besides the calcium content of the bone matrix.

This research was supported partly by a grant from "Consiglio Nazionale delle Ricerche" and partly by a grant from "Ministero della Sanità"

This could occur in otosclerosis. In fact, in otosclerotic bone the number of osteocytes/mm^3 has been evaluated to be around 110 000 compared with 20 000 in lamellar bone and 50 000 in non-otosclerotic fibrous bone (Frost, 1962) The lacunar volume is 0.9% in lamellar bone as against 11% in otosclerotic bone (Frost, 1962)

For this reason we felt the need to check our microradiographic data using a method not influenced by the presence of the osteocyte lacunae. We chose microhardness testing, which was developed by Carlström (1954) and Amprino (1958) to analyse the degree of mineralization of the interlacunar matrix of bone tissue.

MATERIAL AND METHOD

In the present study 9 stapedes from cadavers with normal temporal bones and 18 stapedes or fragments removed during stapes surgery for relief of clinical otosclerosis were examined. The specimens, fixed in 95% alcohol were carefully dehydrated and embedded in methacrylates. To avoid any interference of the embedding medium hardness, the same methacrylate was used and polymerisation *simultaneously carried out in all the specimens.* After polymerisation the specimens were ground on one side. The structures to be tested were selected on the polished surface

with reflected light at an enlargement of ×100 using the Leitz Durimet microscope. The microhardness was tested on the polished surface by applying a square-based pyramidal diamond indenter with angles of 136 (Vickers pyramid) The microhardness expressed in kg/mm² was computed from the formula HV = 1 8544xL/d^2 where L is the load in grams and d the mean length in μm of the diagonals of the imprint. A load of 15 g was always used The diagonals of the imprints were measured by means of a screw micrometric eye-piece at an enlargement of ×400 Following Amprino s (1958) suggestions, imprints were made in the interlacunar matrix at a distance of at least one and a half times the length of their diagonal from any edge of the sample cavities or of any other imprints. When all the imprints had been made and their diagonals measured and recorded a microphoto was taken under vertical illumination In these photos the imprints appeared as black squares over a grey background (Figs. 1 *D* 1 *E* 2 *D* 3 *D*) The specimens were then carefully ground on the surface opposite to that previously tested to a uniform thickness of 40 μm and a microradiograph made (Figs. 1 *A* 1 *B* 2 *A* 2 *B* 3 *A* 3 *B*) with a Machlett type AG 50T X ray tube (7 8 kV filter 1 mm Be). Each section was then decalcified and examined under polarised light using the Leitz Ortolux microscope equipped with Xenon XBO 162 Osram lamp and Berek and Brace compensators (Figs. 2 *C* 3 *C*)

RESULTS

Normal stapedes

Table I shows the microhardness values obtained in the branches and footplate of the normal stapes. Each value represents the mean of 40 determinations in the branches and of 10 in the footplate. In the normal footplate the imprints were made only on the bone at the tympanic surface and not on the calcified cartilage which is present on the vestibular aspect of the stapes.

Table I *Microhardness values in normal stapedes (kg/mm²)*

No.	Age (years)	Sex	Branches	Foot plate
312	8th month fetus	♂	99	96
372	7	♂	133	121
374	20	♀	130	121
324	29	♂	96	118
375	36	♂	98	106
354	48	♂	92	99
369	57	♀	112	113
352	65	♀	128	131
360	70	♀	110	109

From Table I it can be observed that there is no significant change in the microhardness related to age Only in the fetus did the microhardness values seem to be less but, due to the small number of ossicles studied, this observation cannot be taken into consideration.

Fig 1 (*B*) Microradiograph of an otosclerotic stapes of a 15-year-old patient (16). The outlined areas show the transitional zones between the focus and the normal branch. In (*A*) and (*C*) these areas are shown at a higher magnification note that the focus has a lower X-ray absorption (185). In (*D*) and (*E*) the same areas are seen at the same enlargement under vertical illumination. The imprints of the Vickers pyramid are larger in the matrix of the pathological bone

Fig. 2. (*A*) Microradiograph of an otosclerotic stapes of a 44-year-old patient (20). (*B*) The microradiograph at a higher magnification (225) of the area outlined in (*A*) shows a different degree of mineralization in the normal branch and in the otosclerotic focus. The same area is seen in (*C*) with polarized light and in (*D*) under vertical illumination (×225). The imprint of the Vickers pyramid is larger in the matrix of the pathological bone.

Fig 3 (*A*) Microradiograph of an otosclerotic stapes of a 55-year-old patient (×20) The area outlined is shown in (*B*) at higher magnification (225); this includes the transitional zone between the normal branch and the otosclerotic focus. In (*C*) and (*D*) the same area is seen, respectively with polarized light and under vertical illumination (×225). The imprints of the Vickers pyramid are larger in the focus than in the normal branch

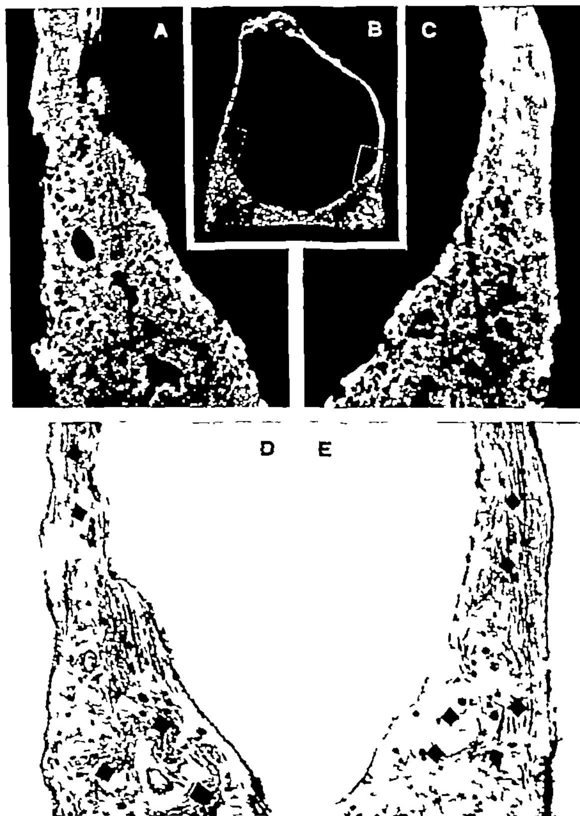


Fig 1

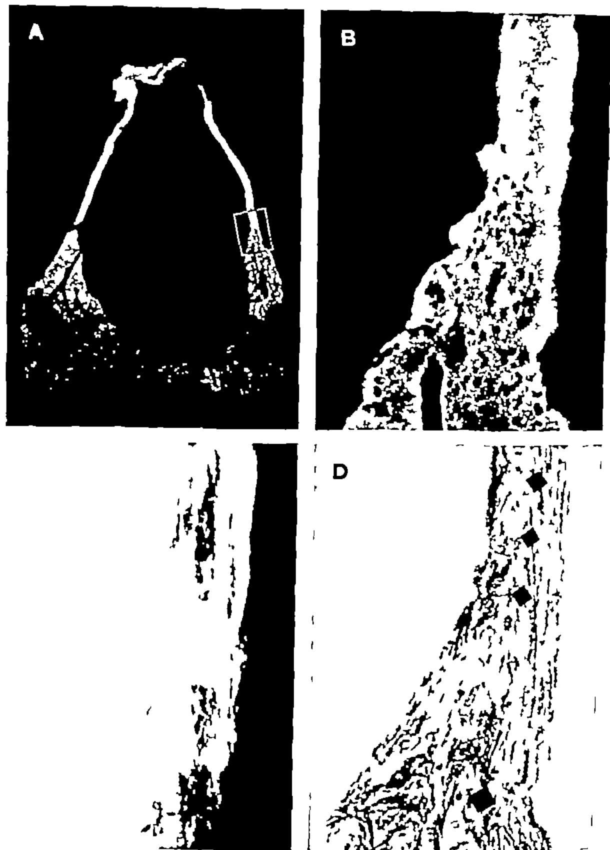


Fig 2

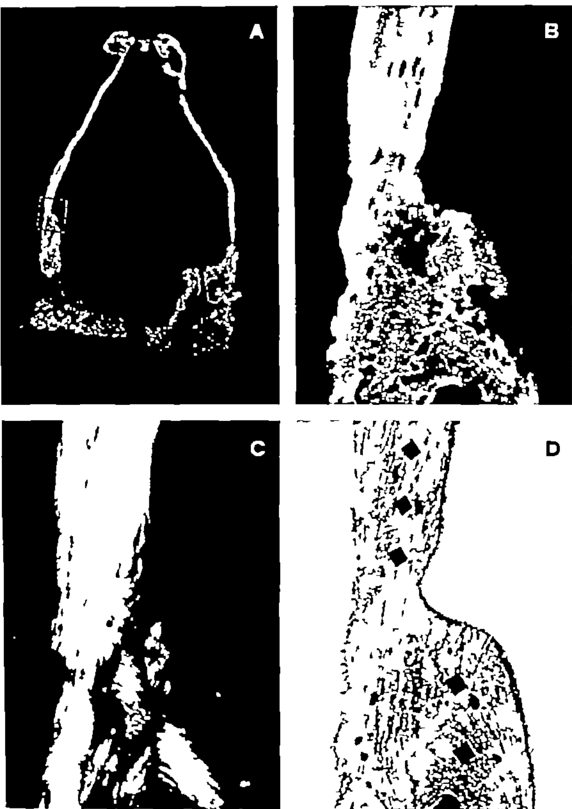


Fig. 3

Table II *Microhardness values in stapedes with "active" otosclerotic foci (kg/mm²)*

No.	Age (years)	Sex	Branches	Focus
289	14	♀	128	109
401	26	♂	[illegible]	76
285	[illegible]	[illegible]	115	99
387	29	♀	119	104
279	35	♀	116	99
400	43	♀	117	105
261	52	♀	100	90
247	57	♀	111	96
402	59	♂	111	101

Otosclerotic stapedes

The otosclerotic stapedes were sub-divided into three groups according to the "activity" of the focus. This evaluation was based upon the dimensions of the marrow spaces, the number shape and dimensions of the lacunae and the characteristics of the bone matrix. In Table II are presented the microhardness values obtained in the active otosclerotic foci and in the corresponding branches. In Tables II III and IV each value represents the mean of 20 determinations in the branches and of 20–30 in the focus.

n the "active" foci the microhardness values ed appreciable differences in the various nts examined. Although a few imprints had dimensions near to those in the normal parts, many of the microhardness values of the "active" foci were lower than those of the normal parts. The mean microhardness value was 10–15% lower in "active" foci than in the corresponding branches.

Table III *Microhardness values in stapedes with "less active otosclerotic foci (kg/mm²)*

No.	Age (years)	Sex	Branches	Focus
265	21	♀	104	97
399	37	♂	103	94
394	44	♂	87	81
254	47	♂	97	90
397	53	♂	87	81
299	65	♀	95	89

Table IV *Microhardness values in stapedes with "quiescent" otosclerotic foci (kg/mm²)*

No.	Age (years)	Sex	Branches	Focus
279	35	♀	116	114
245	41	♀	101	98
395	55	♀	113	[illegible]

Table III shows the microhardness values obtained in "less active" otosclerotic foci and in the corresponding branches. Also in the "less active" foci there was a certain degree of variability in the dimensions of the imprints. The mean microhardness value was 6 7–8.7% lower in the "less active" foci than in the corresponding branches.

The microhardness values of "quiescent *foci are presented in Table IV In the* quiescent" foci the dimensions of the imprints were more or less constant, which means that the mechanical resistance of the bone matrix was rather homogeneous. The mean microhardness value was 1.2–4% lower than in the corresponding branches

DISCUSSION

A strict relationship exists in bone tissue between microhardness and mineralization as it is known that microhardness is higher in high calcified bone (Carlström 1954 Amprino, 1958)

Besides the degree of calcification there are two other factors which can influence the microhardness of bone tissue: (1) the amount of water in the matrix and (2) the denseness and arrangement of collagen fibers (Amprino, 1958) Amprino found that in the parallel-fibered bone, microhardness was 20–25% lower when tested in a plane perpendicular to the axis of the fibers than when tested in a parallel plane.

In our experimental conditions, the first factor mentioned by Amprino can be disregarded, as our material was completely dehydrated and embedded in methacrylates. To

be sure that the methacrylate did not influence our results we tested the microhardness of the resin used and found imprints with a diagonal length in μm more than the double than that in bone tissue. We concluded that the methacrylate did not influence the microhardness values and this opinion is shared by other authors (Marotti & Favia, personal communication).

The denseness and arrangement of collagen fibres should be considered. However according to Blaimont (1968), this factor does not significantly influence bone microhardness values. In the stapes, collagen fibres run perpendicular to the plane tested in the branches or at least, in their non-otosclerotic part (Figs. 2 *C* 3 *C*) Therefore, the higher microhardness values we found at this level cannot, in any way be interpreted as an effect of collagen fibres orientation. The otosclerotic focus, when examined under polarized light, revealed the warp and woof pattern which is characteristic of the fibrous bone. Also at this level, the low values of microhardness we constantly found cannot depend upon the collagen orientation.

We can conclude that the lower microhardness values observed in the otosclerotic foci reflect a lower degree of mineralization which confirms our previous microradiographic studies. We observed a difference in microhardness between active and quiescent foci. The active foci showed lower values which means that they are less mineralized and during the course of their evolution they increase their mineral content.

In Spain stapedectomies were carried out by L. Garcia-Ibañez and in Italy by E. Bocca and S. Iurato.

ACKNOWLEDGMENT

We would like to express our thanks to Professor R. Amprino and Professor G. Marotti for their co-operation.

RÉSUMÉ

La microdureté de 18 étriers otospongieux et de 9 étriers normaux a été étudiée afin d'analyser l'état de leur minéralisation. L analyse a été accomplie a ec un Leitz-Durimet sur des étriers fixés en alcool et inclus en méthacrylate On a trouvé que l'os otospongieux est moins minéralisé que l'os normal. Le contenu minéral du foyer actif est inférieur de 10 à 15% à l'os normal tandis que le foyer en repos est moins minéralisé de 1.2 à 4% On a comparé les données de la microdureté avec celles obtenu par la méthode microradiographique.

ZUSAMMENFASSUNG

Untersuchung der Mikrohärte zur Analyse des Mineralisationsgrades an 18 otosklerotischen und 9 normalen Steigbügeln. Die Gewebeproben — in Alkohol fixiert und in Metacrylat eingebettet — wurden mit dem Leitz-Durimet getestet. Dabei wurde ein im Vergleich zu einem normalen Knochen verminderter Mineralisationsgrad des otosklerotischen Knochens festgestellt. Der Mineralsalzgehalt aktiver Herde ist um 10–15% geringer als derjenige des normalen Knochens; ruhende Herde sind 1.2–4% geringer mineralisiert. Die Mikrohärtewerte wurden mit mikroradiographischen Befunden verglichen.

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DISCUSSION

A E. Kortekangas: Can microhardness testing be applied in very small fragments of stapes? My question arises from the experience of my countryman Pohakka who often got controversial results by microradio-

graphy in attempts to make a mineralization analysis. His studies using mineral analysis by atom absorption spectrophotometry and by neutron activation analysis showed with increasing immaturity a decreasing stage of mineralization of otosclerotic foci. These results were in correlation with his results of the crystalline structure in otosclerotic foci.

I Friedmann. Does your method lead to the study of aetiological problems and to the differential diagnosis of various processes affecting bone e.g. Paget's disease, osteogenesis imperfecta?

S Iurato (Reply) to Mr *Kortekangas.* Using a load of 15 g the length of the diagonals of the imprints was between 15 and 20 μm in our material. This means that microhardness testing can be applied to very small fragments of bone tissue. The only reason to use complete stapedes was to compare the mechanical resistance of the otosclerotic bone matrix with that of the normal parts.

To Mr *Friedmann.* The microhardness testing was developed by Carlström (1954) and Amprino (1958) to analyse the degree of mineralization of the interlacunar matrix of bone tissue. As far as I know all the researches were performed on normal bone tissue. This is the first time that the method has been applied to pathological material.

Acta Otolaryng 73 87-93 1972

TOTAL MYRINGOPLASTY FUNCTIONAL ASPECTS

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Abstract. In recent experiments employing time-averaged holography strong evidence was found supporting Helmholtz' hypothesis of 1868 concerning tympanic membrane action. Since this action requires the tympanic membrane to be tightly coupled to the manubrium and not to be subjected to stretch during its vibratory displacements, attention was focused upon tympanic membranes lacking the characteristic fiber network of *membrana propria*. The displacement patterns of tympanic membranes, 6 to 7 months after replacement (fascia grafts), were recorded in cats, once more using time-averaged holography In general, these tympanic membranes were stiffer than normal ones, and coupling to the manubrium was poor Thus, the maximal displacements occurring in atypical locations involved stretching of the membrane, and there was no indication of the normal transformer action. The combination of high compliance and good coupling found in normal tympanic membranes was thus shown to be facilitated by the intricate fiber network of *membrana propria*.

Last year we presented some new evidence on the displacement pattern of the tympanic membrane (TM) in cats (Khanna, 1970· Tonndorf & Khanna, 1970 1971). This work was facilitated by application of "time-averaged holography" a new technique that is basically an optical interference method (Powell & Stetson, 1965) It permits recording displacement patterns of an entire membrane *in toto* i.e., at the same instant of time, and the subsequent measurement of amplitudes at any point of the membrane with good precision.

Fig. 1 A N"-46 may serve as an illustration of the method and also as a demonstration of the results obtained on a normal TM. The alternating dark and bright lines ("fringes") are destructive and constructive interference lines, each of them representing an *iso-amplitude contour* Amplitude increases as a function of their order number the precise relationship being given by a zero-order Bessel function. On an edge-clamped membrane, such as the TM, the first bright fringe lies invariably at the periphery and order number—and thus displacement magnitude—increases toward the center The holographic reconstruction "N"-46 of Fig. 1 A then indicates that in response to a low frequency the membrane forms two separate displacement maxima, a lesser one anteriorly to and a larger one posteriorly to the manubrium. On the manubrium itself fringes run crosswise, in an oblique direction, parallel to the axis of rotation The displacement amplitude of the manubrium, even at its tip, is invariably less than that of the membrane on either side.

This was precisely the displacement pattern Helmholtz (1868) had postulated. Based upon the "mechanics of curved membranes" he had argued that the relatively large displacements of low force of the membrane would be transformed into lesser displacements of greater force at the manubrium In other words, that the TM constituted an active part of the middle-ear transformer mechanism. As seen in Fig. 1 A, our data strongly support this notion

Two conditions were basic to Helmholtz' concept: (*a*) that the TM be suitably curved—it is known to have the shape of an inverted

This work was supported by several NINDS grants.

A





B





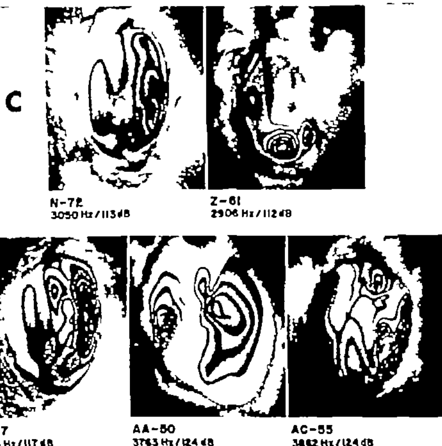


1 Holographic reconstruction of the displacement patterns of the grafted TMs (cats "Z" AA" AC"). At each of the four different frequencies (rows A D) the abnormal patterns are juxtaposed by one normal pattern (cats "N" or "M"). (Tonndorf et al., *Ann Otol*, 1971)

cone with curved crossections—and (*b*) that the membrane must not be stretched during its displacement, lest energy be lost from transmission. Both of these conditions point to the importance of the fiber network of the *membrana propria* for the function of the TM

We were therefore interested in the function of TMs that lack this fiber network. To this end, total tympanoplasties were performed in cats using muscular fascia, and, after waiting periods of 6 to 7 months, their vibratory patterns were recorded. The final series consisted of three animals. A fourth animal, found accidentally was added to the series. In the latter case, the lower third of the TM had no connection to the manubrium, but was found bulging into the ear canal. This TM then had a normal set of fibers, but its curvature and suspension were different from normal.

In contrast to some of our earlier studies (Tonndorf & Khanna, 1970) animals were kept *alive* during the entire experiment. In each animal, fifty to sixty holograms were taken at various frequencies and at a number of intensity levels for each frequency Fig. 1 shows a small selection of the holographic reconstructions on the grafted TMs, one or two per frequency juxtaposed by a pattern obtained on a normal TM at the same frequency There is little if any resemblance between the normal and abnormal patterns of any frequency For one thing, the locations of the



area of maximal displacement deviated considerably from those found in normal TMs. Also, while the normal pattern became complex when the frequency exceeded 3 kHz, with some abnormal TMs, complex patterns were already seen at relatively low frequencies (e.g., AC" 27 2 kHz) with others, they remained simple, even at high frequencies (e.g., AA" 50 3 7 kHz)

In the TM with the loose lower section, the maximal displacement occurred invariably in that section (Fig. 2), as one might have expected, and the whole pattern was distorted accordingly The complete separation of the pattern by a nodal line perhaps already apparent at 3 kHz, but definitely at 4 kHz ("O" 28) was an interesting finding. It suggests that at this frequency the two sections might vibrate in opposite phases, something we have never observed that clearly in any other animal.

There was no simple way of correlating the findings of Figs. 1 and 2 with the appearance of the TMs, i.e., the extent of scarring (Fig. 3) or with the middle-ear transmission losses that were assessed with the aid of cochlear microphonic recordings (Fig. 4) Scarring was more wide-spread in cat "AC" than in cat "AA" yet it was the latter's TM that failed to break up into sectional vibrations at high frequencies. It was only in cat "Z" apparently that the heavy scar in the posterior quadrant reduced vibration amplitude quite drastically in that region. But then again, cat "Z" had the least transmission loss among the tympanoplasty animals. Significantly the three grafted TMs possessed rather shallow cones, having practically straight cross-sections.

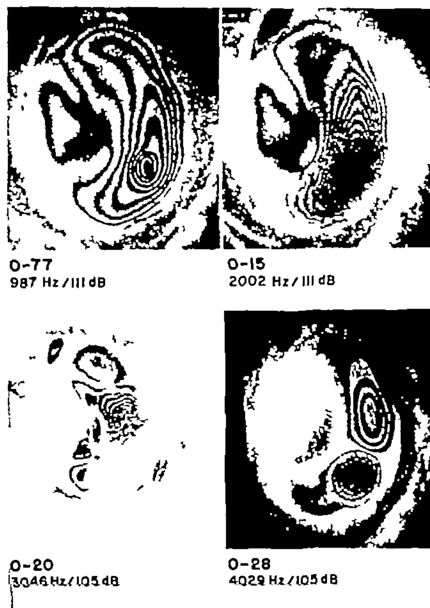


Fig. 2. Holographic reconstructions of the displacement pattern of the TM with the loose lower section (cat "O") for four different frequencies. (Tonndorf et al., *Ann Otol* 1971)

Fig 5 gives the volume displacement for a uniform sound pressure level (100 dB) in one normal TM and in those of the four experimental animals. It is seen that all four experimental TMs showed less compliance i.e., they were considerably stiffer than a normal TM.

This lack of compliance was not the only shortcoming. In normal TMs, the amplitude ratio of maximal membrane displacement, i.e., that in the posterior region, to that of the malleus remained fairly constant from animal to animal Fig 6 gives this ratio once more for one normal animal and the four experimental animals. In the latter case, maximal displacement is given regardless of its location. [Note that in several cases, indicated by arrows, the malleus displacement could no be observed, even at the highest sound pressure levels employed in these cases, the real values must lie even higher than indicated.] Fig. 6 demonstrates that, in all experimental animals, the efficiency of the TM in driving the malleus was rather poor In other words,

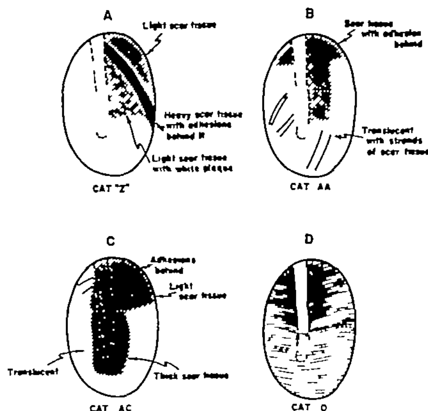


Fig. 3. Appearances of the four experimental TMs at the time of sacrifice: somewhat schematic. (Tonndorf et al., *Ann Otol* 1971.)

coupling between membrane and malleus was relatively loose. Consequently, the membrane displacement must have involved a good deal of stretching, which, as already stated, is detrimental to the transformer action and to energy transmission in general.

Cat "O" (Fig. 2) presented a slightly different problem. Here, the fiber network was

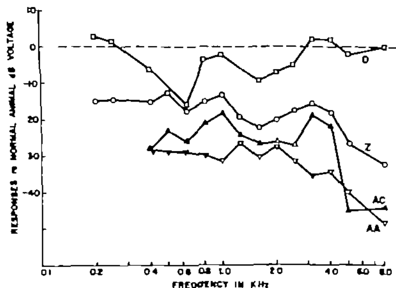


Fig. 4. Transmission losses in the four experimental ears assessed with the aid of cochlear microphonic recordings. (Tonndorf et al., *Ann Otol*, 1971.)

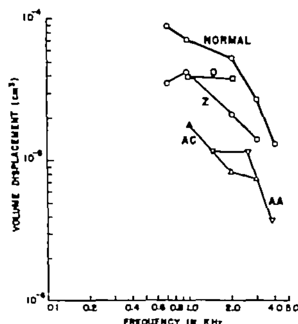


Fig 5. Volume displacements, calculated from holographic data, *vs* frequency at a sound pressure level of 100 dB for one normal TM and those of the four experimental animals. (Tonndorf et al., *Ann Otol* 1971.)

said to be intact, but many fibers were not to the manubrium in the right laces, and also the curvatures in the lower section was much larger than usual. The highest displacement amplitude was always found in the lower section (cf Fig 2) Coupling to the remainder of the TM at least at low frequencies, was reasonably good, since there was a coherent displacement pattern However coupling to the manubrium was once more poor (cf Fig. 6) apparently on account of the excessive curvature, and the fact that the fibers did not connect to their appropriate places. However there was one other point that has to be considered. Fig. 5 indicated that the overall compliance of this TM, in spite of the loose lower section was still less than normal. Closer inspection showed that the upper normal-appearing part of this TM vibrated actually at less than normal amplitudes. This finding can be explained by the fact that the lower section, with its excessive amplitude, acted very much like a perforation and imparted a sizable amount of sound pressure to the tympanic cavity this sound pressure, being in proper phase, partly cancelled the displacement of the upper part

The findings in cat "O" indicated that the low efficiency of TM action found in the grafted membranes was not exclusively due to the scar formation that appears to be an unavoidable shortcoming in cats.

An earlier experiment (McArdle & Tonndorf 1968) had given evidence to the good coupling obtained in normal TMs. Driving the TM by a needle-shaped vibrator in the posterior quadrant had resulted in the same efficiency of transmission as driving the manubrium directly provided the 3 1 amplitude ratio normally found between these two places was taken into account. Thus, coupling to the manubrium was excellent from the indicated place, although it was not that good in the inferior quadrant. Then, with the vibrator in place in the posterior quadrant, about half-way along the manubrium, a small incision was made between the point of contact and the manubrium There was no change as judged

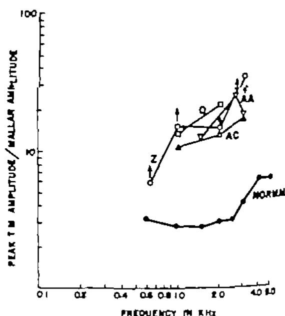


Fig 6. The ratio: maximal displacement/mallar displacement, for one normal TM (posterior quadrant) and those of the four experimental animals regardless of location (Tonndorf et al., *Ann Otol* 1971.)

from cochlear microphonic recordings. In fact, there was no noticeable change until the incision had been lengthened to almost the full length of the manubrium, indicating that coupling was good throughout the membrane in any direction.

In all experimental TMs, then, there were sections that had good compliance (i.e., good sensitivity), but these were poorly coupled to the manubrium. Other sections were stiffer and, when suitably located, better coupled to the manubrium. However their sensitivity was generally poor It was only in normal TMs with their characteristic fiber network and proper curvatures that good compliance (that is, high sensitivity) was combined with good coupling to the manubrium (that is, there was no membrane stretching with its attendant loss of transmission) Both of these conditions are prerequisites for proper transformer action.

RÉSUMÉ

Dans des expériences de longue durée des membranes tympanales furent remplacées par des greffes de fascia. Leurs formes de déplacement, déterminées grâce à la méthode holographique avec moyenne temporelle, de même que l'atténuation due à l'oreille moyenne révèlent la signification de la trame des fibres du tympan normal.

ZUSAMMENFASSUNG

Trommelfelle von Katzen wurden in langzeitigen Experimenten durch Fascie ersetzt. Ihr Auslenkungsmuster bestimmt mittels zeitlich-gemittelter Holographie, sowie der Übertragungsverlust wiesen auf die Bedeutung des charakteristischen Fasernetzwerkes normaler Trommelfelle hin.

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DISCUSSION

R. Hinchcliffe: Could you comment on the results of a study recently conducted at the Institute of Laryngology and Otology in London. This covered the results of myringoplasty using both audiometric and acoustic impedance measurements by my colleague Dr Booth. A statistical analysis which I did on the data showed no correlation between the compliance results (either overall middle ear compliance or gradient of the middle ear compliance) and the audiometric results. Could Mr Tonndorf please comment in the light of his "time-averaged" holographic studies?

J Tonndorf (Reply) to Mr *Hinchcliffe:* You will recall that $z = p/v$. Thus, our volume displacement measurements can be readily expressed in terms of impedance. In our case, too, the correlation between findings, e.g., volume displacement to transmission losses were poor They were somewhat better for membrane displacement to mallear displacement. With respect to transmission, a number of subtle items (scarring, adhesions in the middle ear etc.) must be taken into account and one can simply not predict the outcome on the basis of compliance measurements. Clinically the dB scale helps, since a 50% loss in *physical* transmission amounts to only −6 dB!

Acta Otolaryng 73 94–103 1972

ANALYSIS OF FLOW PATTERN IN VAS SPIRALE

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Abstract. Microcirculation to the organ of Corti resembles that in terminal capillaries of other parts of the body but in this case, because of the limited number of vessels, cessation of flow becomes important to the function of the organ of Corti. Motion picture analysis at rapid-framing rates reveals the flow pattern and its relation to the structures of the basilar membrane and tunnel of Corti. These are to be presented with an interpretation of the significance of this capillary circulation.

The *vas spirale* first named by Huschke (1844), and described in detail by Corti (1851) can no longer be regarded as an appendant to the basilar membrane. Indeed, it can no longer be said that the organ of Corti does not have a blood supply for evidence from both the geometrical arrangement and the physiological function indicate this capillary to be complex in its branching and to have an intimate relationship to the fluid spaces between the cells of the sensory epithelium.

The basilar membrane, the organ of Corti, and these capillaries are so constructed and situated that fluid exchange is facilitated. In guinea pigs, primates and other mammals, the feet of the inner pillar cells rest on the edge of the spiral lamina, between the *habenula perforata* and the attachment of the basilar membrane From this attachment to a point beneath the outer pillar there is only a thin fibrous layer separating the fluid space of the tunnel and the lamella cells on the scala tympani side.

Research supported by The John A. Hartford Foundation and PHS grants NS-03410 and NS-05785

Just beneath the outer pillar cell the fibrous layers become double, thickening and strengthening the basilar membrane from that point to the attachment at the spiral ligament. Corti (1851) described these two zones as the *zona arcuata* and the *zona pectinata* and we have recently (Lawrence, 1971 *b*) pointed out, in specially prepared histological sections, the relationship between the thin area beneath the tunnel and the spiral capillaries.

As shown in Fig. 1 these capillaries emerge from the spiral lamina to form, roughly two parallel systems lying beneath the feet of the pillar cells, thus bordering the tunnel. The vessels of this dual system have been labeled by Axelsson (1968) the vessel of the basilar membrane (*V as membranae basilaris*), and the vessel of the tympanic lip (*V as labii tympanici*) There may be occasional gaps in these vessels but one or the other or both are there in sufficient prominence to provide fluid exchange

As a matter of fact, these capillaries lie closer to the sensory cells than do capillaries in some other parts of the body to the cells which they support. Friedman (1969) points out that in certain species, including man, only the inner layer of the retina contains blood vessels, leaving an avascular area of width varying from 100 to 200 μm. He further calls attention to the high degree of efficiency of these capillaries in supplying oxygen to rods and cones over a distance of at least 50 μm.

Between neurons of the brain and the capil-

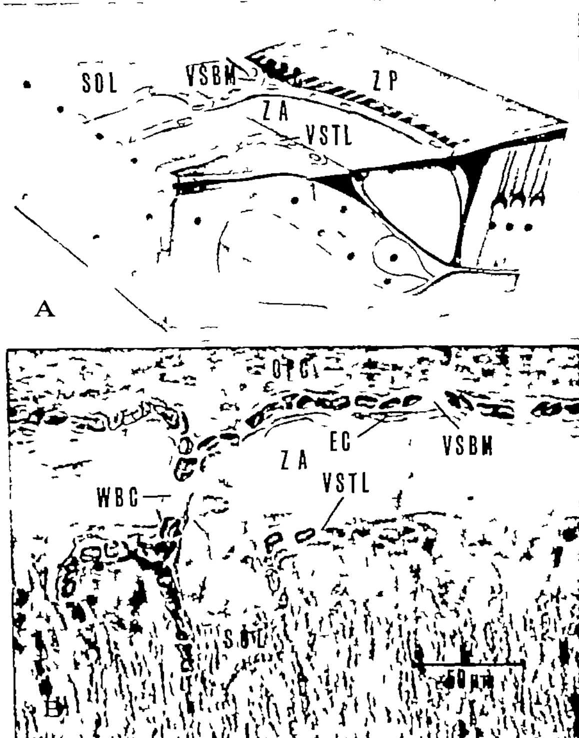


Fig. 1. The spiral capillaries of the *zona arcuata* beneath the tunnel of Corti. (A) Schematic drawing to show the relationship between the spiral capillaries and the fluid spaces of the organ of Corti. (B) Photomicrograph of the scala tympani side of the basilar membrane from a normal unoperated guinea pig to show capillaries comparable to those shown in (A). *EC* endothelial cell; *OPC* outer pillar cell, *SOL* spiral osseous lamina; *VSBM* spiral vessel of basilar membrane; *VSTL* spiral vessel of tympanic lip; *WBC* white blood cell; *ZA* zona arcuata, *ZP* zona pectinata.

lanes lie the glial cells which, Nakai & Hilding (1967) emphasize, is a situation similar to that of the supporting cells of the organ of Corti lying between the spiral capillaries and the hair cells. Their study was designed to locate the enzyme adenosine triphosphatase which plays an essential part in the transport of fluid components across membranes, and they call attention to the observation that both the glial cells and Deiters cells demonstrate enzyme activity

The distance between the spiral capillaries and the hair cells is generally less than 50 μm while the distance from capillary to tunnel fluid is only a few microns.

There are experiments demonstrating that these spiral capillary vessels are essential for the supply of oxygen to the organ of Corti. Okumura (1970) perfused glucose-glucoseoxidase mixture into scala tympani a procedure which reduces the oxygen tension in the mixture as glucose is oxidized This perfusion, however could not reduce the cochlear a.c. potential more than 6 dB unless at the same time the supply of oxygen through the blood diminished The capillaries provide a con ious supply of oxygen so that depletion of in perilymph does not have a maximum effect on the action of the organ of Corti i.e on the generation of an a.c potential. This implies that perilymph freely pervades the spaces of the organ of Corti and there is convincing evidence that this is so

Vosteen (1970) has reviewed the evidence coming from his laboratory in a most excellent and comprehensive paper When thorium dioxide particles are injected into the scala tympani (Ilberg & Vosteen, 1969) they are found to penetrate all of the intercellular spaces of the organ of Corti, whereas these particles, when injected into the scala media do not penetrate beyond the reticular lamina. One must conclude, with Ilberg and Vosteen that the two fluids, endolymph and perilymph, are related to each other through the transport properties of Reissner's membrane and that the fluids occupying the spaces of the organ of Corti differ from perilymph only by the chemica contributions of the spiral capillaries. Unti recently these capillaries have received littl attention but following the observation (Lawrence, 1966) that the cellular components of the organ of Corti depend upon the continuous flow of blood provided by the spiral vessels, a technique for studying the flow through the vessels by motion pictures was developed (Lawrence, 1970 1971 a)

Flow analysis by motion picture

Although there are many characteristics of microcirculation that appear as common to capillary systems in general, one can never know whether or not these apply to a particular organ until that organ is studied. However especially in the study of the ear it is extremely difficult to be sure that the surgical approach to the microcirculation does not alter or modify the flow under study but by very careful dissection in the guinea pig, without disturbing the arterial supply or the venous drainage the flow through the spiral vessels can be observed and photographed.

Our surgical approach and positioning of animal and motion picture camera have been described (Lawrence 1971 c) As shown in the earlier reports the flow through the vessels is extremely rapid for a terminal capillary and the ordinary framing rate of motion pictures does not slow the visual portrayal of the flow sufficiently to permit a study of the behavior of the cellular components or the characteristics of the capillary wall In order to obtain rapid framing rates along with high-power magnification and still provide enough light, the Chadwick-Helmuth point-source Strobex has been used. This light source is triggered by the camera frame-drive motor so that the strobe flashes at the exposure of each frame. Framing rates up to 128 frames per second with a magnification through the microscope of $\times 750$ had been obtained but many difficulties have been encountered.

Even with short-flash duration the tissue retains heat which builds up so exposure dura-

tions have to be kept short There was a tendency for the flash to be inconsistent in intensity The present motion picture reflects this problem and sections have been chosen where the effect is minimal. Because of the size of the light, positioning it close to the surgical area is difficult and an optical flare often results, especially with the high-power objective Because of these difficulties this type of lighting will no longer be used.

Observations from motion picture

Our present purpose has been to describe the normal blood flow through these capillaries. However it is difficult to determine exactly what is normal. The capillaries are very sensitive to alteration in their environment and unless the fluid covering them is kept constant in amount and temperature and mechanical manipulation kept to a minimum, different areas of the capillary may react differently

The motion picture that accompanies this paper demonstrates the various alterations observed in the flow of blood. The most remarkable characteristic of this flow is its velocity Earlier (Lawrence 1971 *a*) we had estimated this to be in excess of 320 μm per second With the high framing-rate camera it is possible to be considerably more accurate. The velocity of flow was determined by measuring the size of a red cell in the projected image, accepting 7 5 μm as the average size of a guinea pig erythrocyte, and measuring the distance a cell travels from frame to frame Rather consistently from animal to animal, the normal flow rate averages 1 75 mm sec. Costa & Brånemark (1970) give 800 μm/sec for the vessels of the stria vascularis while Perlman et al. (1963) give 149 μm/sec as the normal average flow velocity in the vessels. In the experiment of the last author this velocity could be increased to 535 μm/sec by an injection of 5 μg per kg adrenalin directly into the carotid. Similar injection in our animals increased, immeasurably the flow velocity in the spiral capillaries.

However the spiral capillaries are highly susceptible to changes in the local environment. Certain ototoxic drugs (Lawrence, 1970), too much acoustic stimulation (Lawrence et al., 1967), or actual irritation with a cotton fiber may cause the flow to stop

Four different types of flow are demonstrated in the motion picture Normal flow is smooth, rapid and continuous but spontaneous variations, the cause of which is not obvious, do occur

The flow in one branch of a "T" junction may reverse its direction rather suddenly This, of course, occurs in other areas of microcirculation probably produced by the closing down of some vessel in the network causing the flow to alter its route This may occur in the spiral capillary network but the field of view is small, so that only a limited portion of the capillary chain can be seen

Similarly the flow in one branch may pulsate with the heart beat, while flow in the other branch continues in an uninterrupted manner Although the pathway to the larger vessels is relatively short, how this pulsation can be produced in one branch and not the other is not obvious.

Occasionally a white cell will tend to obstruct the flow especially at a junction. This produces a gap filled with plasma. Two or three white cells have been seen to jam up at a junction slowing or stopping the flow and at one point in the motion picture such an obstruction is seen holding up the flow for a short period and then suddenly breaking free. These events occur in all animals regardless of experimental procedure and have been reported for the vessels of the stria vascularis (Costa & Brånemark, 1970)

Because these events are fleeting and individual frames of the motion pictures do not show clearly the blood cells and the cell wall, special preparations were made of the vessels and basilar membrane to give a clearer picture The basilar membrane including the capillaries in the area viewed by the microscope and motion picture camera, were fixed directly with formaldehyde. The area was dis-

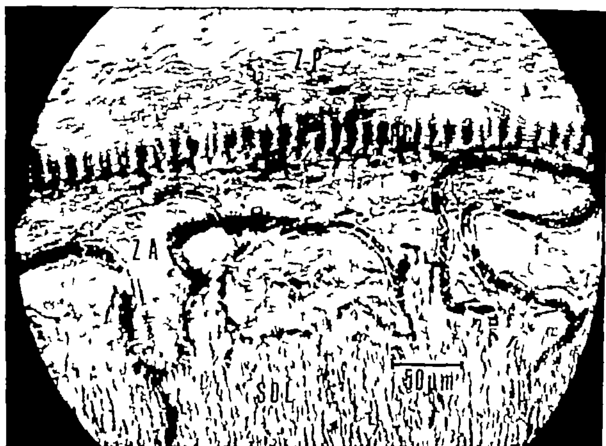


Fig. 2. Photomicrograph of the basilar membrane the scala tympani side to show the occasional ing and interconnections between the two sets of illaries. At the left is a region where the two capillaries become one, following a path along the middle of the *zona arcuata* beneath the tunnel. *SOL* spiral osseous lamina, *OPC* outer pillar cell; *ZA* *zona arcuata* ZP· *zona pectinata*. OP 1291

sected out, placed upon a slide and photographed through the microscope

Observations from histology

Fig. 2 is a relatively low power view of the basilar membrane from the scala tympani side to show the occasional loops and interconnec tions of the capillaries that may occur In the upper portion of the photomicrograph many tympanic lamella cells can be seen Below these in the illustration are the feet of the outer pillar cells and the base of Deiters cells. It is at this point where the *zona arcuata* becomes thickened by a fibrous layer to form the *zona pectinata*. The capillaries lie within the area of the *zona arcuata* and a pericapillary space can be seen around the left-hand capillary The granules appearing along the walls of the pericapillary space are not uncommon, but their significance is unknown.

A clear view of a white blood cell is seen in Fig. 3 There is a gap between the white blood cell and the red cells which are bunched up in the junction between the vessel of the tympanic lip at the lower edge of the photomicrograph and the branch which makes a "T" junction with the vessel of the basilar membrane Variations in capillary diameter are clearly seen in this photomicrograph. An extremely narrow part occurs in the left-hand branch, while the vessel is fairly wide just above the white cell The presence of white cells and changes in vessel diameter are charac



Fig. 3. A typical "T" junction showing the connection of the basilar membrane capillary (*VSBM*) with that of the tympanic lip (*VSTL*) in an operated ear. A white blood cell (*WBC*) has slowed down the passage of red cells and produced a plasma gap. Of particular note is the variation in vessel diameter obvious in the photomicrograph. *SOL*, spiral osseous lamina, *ZA*, *zona arcuata*, *ZP*, *zona pectinata*. GP 1304

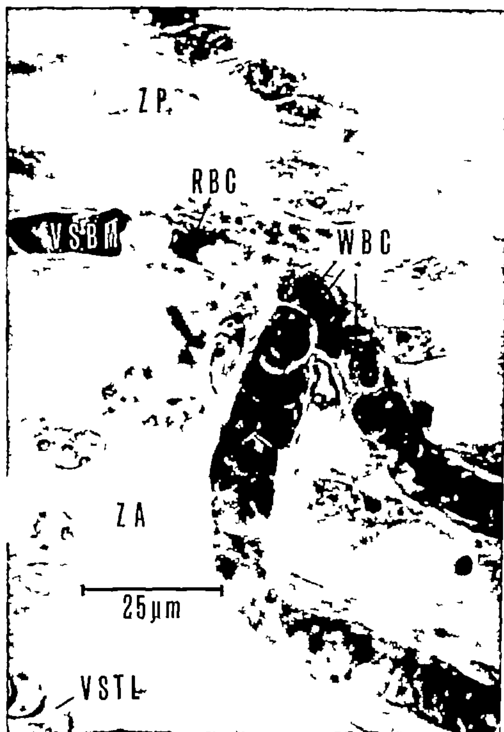


Fig 4 Photomicrograph of another type of "T" junction showing the jamming of cells caused by the pressure of three white cells (*WBC*) passing at the same time. There is also an obvious narrowing of the vessel with a misshapen red cell (*RBC*) passing through. Same animal as Fig. 3 *VSBM* spiral vessel of basilar membrane· *VSTL* spiral vessel of tympanic lip· *ZA* zona arcuata *ZP* zona pectinata. GP 1304.

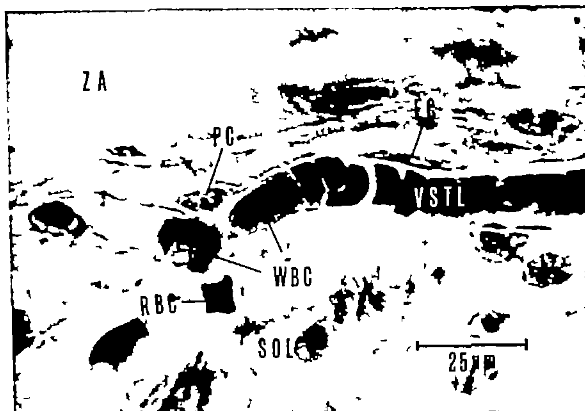


Fig. 5. Photomicrograph of the vessel of the tympanic lip (*VSTL*) in the unoperated ear of GP 1304 showing the same grouping of white cells (*WBC*) at a "T" junction with a distorted red cell (*RBC*) in the lower branch. *EC*, endothelial cell, *PC* pericyte *SOL*, spiral osseous lamina; *ZA* zona arcuata.

teristic of all ears examined. This particular photomicrograph is from an unoperated ear

A similar picture can be seen in the operated ear of this same animal. In Fig. 4 a group of these white cells crowding a "T" junction are seen. It is obvious that the flow is momentarily blocked with narrowing of the vessel diameter in the left hand branch. A red cell has changed its shape in squeezing through this narrow space. On the other hand the vessel is much larger in the branch leading to the "T" and is packed with cells.

The same sort of red cell distortion is seen in the photomicrograph of Fig. 5 Again this is from an unoperated ear and the direction of flow appears to be toward the bottom of the picture. The white cells are crowding at the T" junction and the capillary is narrow where the red cell appears to have altered its shape.

Although the flow through these capillaries is so rapid that even high framing rate motion pictures do not make cell types obvious, single frame analysis shows the presence of white cells in addition to the numerous red cells. The difficulties the white cells have in getting through these narrow capillaries is obvious. Changes in vessel diameter are also apparent.

CONCLUSIONS

The *vas spirale* is not a simple capillary appendant to the basilar membrane, but is a complex dual system consisting of vessels along the tympanic lip of the spiral osseous lamina and of vessels coursing along the *zona arcuata* side of the outer pillar cells. There are many loops and junctions between these two sets of capillaries hugging tightly to the scala tympani side

of the thin single fiber layer of the basilar membrane beneath the tunnel of Corti.

High framing rate motion picture photography reveals the flow velocity within this capillary system to equal the fastest occurring anywhere in the body for this type vessel 1 75 mm per second. The capillaries are ex tremely sensitive to local environmental in fluences, demonstrating changes in diameter and exhibiting considerable versatility in flow characteristic.

RÉSUMÉ

La microcirculation vers l'organe de Corti ressemble à celle des vaisseaux capillaires terminaux des autres parties du corps; mais dans le fonctionnement de l'organe de Corti l'arrêt du flot joue un rôle important à cause du nombre limité des vaisseaux. L analyse de film accéléré révèle les rapports de la circulation avec les structures de la membrane basilaire et le tunnel de Corti. Cette présentation sera suivie d une interprétation de la signification de cette circulation capillaire.

ZUSAMMENFASSUNG

Die Mikro-Zirkulation zum Cortischen Organ ähnelt dem, in den terminalen Kapillaren anderer Körper aber in diesem Falle wird ein Aufhören des stromes, wegen der begrenzten Anzahl von Ge wichtig für die Funktion des Cortischen Or Eine Film-Analyse mit Schnell-Rahmung (ra-d-framing rates) zeigt das Blutstrom-Schema und dessen Verhaltnis zu den Strukturen der Basilarmembran und des Cortischen Tunnels; dieser Film soll mit einer Interpretation der Wichtigkeit dieser Kapillar Zirkulation vorgefuhrt werden.

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DISCUSSION

M Portmann. La communication de M Lawrenc permet d expliquer très certainement des nombreu aspects de la pathologie otologique. Ne pensez vou pas que dans certains cas les changements de direc tions observées dans le courant sanguin sont l résultat d'un effet stroboscopique entre la fréquenc de passage des cellules et la fréquence de prise d vue du film?

S K Bosher· Could you give us some more detail about your observations. Lanthanum studies hav revealed the basilar membrane capillaries to be of th usual type concerned with extra-cellular fluid exchang and consequently the fluid within the tunnel an other tissue spaces of the organ of Corti woul constitute a relatively large reservoir to compensat for short periods of vascular obstruction, despite th unusual and unexpected rapidity of the circulation i this region. In this respect it would be interesting t learn the possible durations of the cessation of th blood flow demonstrated in these vessels.

M Lawrence (Reply) to Mr *Portmann*. The reversa

of flow is not unique to the microcirculation of the ear: it has been observed universally in studies of microcirculation. We have observed it at all framing rates and with steady light illumination. The reversal of flow is not due to a stroboscopic effect.

To Mr *Bosher:* The obstructions to the blood flow may vary from short periods to an indefinite period depending upon what has caused it. Sticking white cells may produce a very short period obstruction while a swollen endothelial cell produced by an ototoxic drug or loud sound may produce a long-time obstruction. These capillaries play an important part in providing nutrients and oxygen to the fluids within the spaces of the organ of Corti. Experiments being carried out now indicate that the supply must be fairly constant.

Acta Otolaryng 73 104–111 1972

POSTUROGRAPHY AS AN AUXILIARY IN VESTIBULAR INVESTIGATION

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Abstract Posturography the recording of the way in which man stands upright, proves to be a useful auxiliary in vestibular investigation. The authors divide this posturography into "spot stabilometry" (or statokinesimetry SKG) and "linear stabilometry" (or stabilometry STG). Special attention is paid to the linear stabilometry (STG). The low frequency-sway of ca 0.2 Hz is nearly pathognomonic for vestibular stimulation. In the spot stabilogram (SKG) the area of the spot and the place of the spot in relation to the centre are of interest.

The weight of man varies between 50 and 100 kg and his length between one-and-a-half and two metres. This tall and relatively heavy body stands upright on a polygonal base of more than about 300 square centimetres. rography the theory of standing upright, therefore of importance for physiology and pathology and in certain circumstances for the physiology and pathology of the vestibular apparatus. The latter subject is our field of interest.

Man stands upright because his muscles, his joints and his skeleton are conditioned for it. These factors together are called the kinetic or static system. This apparatus is in normal circumstances and in normal persons mainly governed by proprioceptis. Normal persons, especially trained persons, can stand upright by making only very small excursions controlled by proprioceptis. The visual apparatus and the vestibular apparatus hardly play a part in normal circumstances. Under *abnormal* circumstances, i.e in experimental, investigational and pathological circumstances, the vestibular system can manifest itself in a standing person. We think here for instance o the Romberg test.

The method of posturography and its modifications has already been used many times by biophysicists (Nashner, 1970 Thomas & Whitney 1959) ophthalmologists (Baron et al, 1968) physiologists (Joseph & McColl, 1961 Begbie, 1967), and otolaryngologists (Kitahara 1965 Aubry & Pialoux, 1968) Henriksson et al. (1967) used this method, paying special attention to the Romberg test. Recently Njiokiktjien published a very complete monograph on this subject with special reference to neurologic patients.

The principle of posturography (statokinesimetry and stabilometry) is shown in Fig. 1 *a*. The patient stands on a round platform, the centre of which is fixed on a steel bar This bar is so strong that the patient does not observe its very slight bending when standing on the platform. Along this bar four strain gauges are attached at right angles. The opposite strain gauges (180) are coupled via a Wheatstone bridge (Fig. 1 *b*)

1 The excursions of the spot on the storage-screen are recorded for 30 seconds and photographed (Fig 2).

2. The backward-forward excursions and the left-right excursions are simultaneously registered on a time-base of 1 cm to 2 seconds (Fig 2) We called the spot, according to Baron et al., *statokinesigram* (SKG). The two

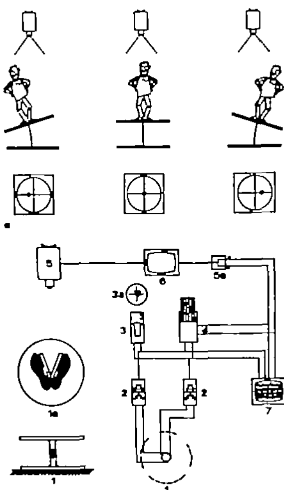


Fig. 1. (*a*) Principle of posturography. (*b*) Block diagram, *1, 1 a*, platform, *2*. Wheatstone bridge and amplifier. *3, 3 a*. storagescope; *4*. writer. *5*. TV camera. *5 a*, *XY* divider. *6*. monitor. *7*. scope.

curves on time base (backward-forward and left–right direction) we called *stabilogram* (STG).

Physical properties of this procedure:

1. Concerning the reproduction of real excursions. The curves do not reproduce exactly the excursions of the point of gravity, but are *very closely* related to it.

2. Physical qualifications of our apparatus. (*a*) Linearity within the reading exactness of the registration apparatus. (*b*) The output is independent of the velocity of displacement and indicates exactly the reaction of the forces on the platform. (*c*) Independence of frequency in the range of 0–25 Hz.

For the investigation of head-movements (see second half of this paper) we extended our apparatus with the following equipment. A light is attached on the head of the patient and via a television circuit the movements of the head are registered on a TV monitor. Besides this, X and Y displacements of the spot are transformed in two pen-written curves in the same way as in body-stabilometry (head-stabilometry). So we get four curves, two of the original body stabilometry and two of the head stabilometry. This TV circuit gives a linear response up to 8 Hz.

The stabilograms of a normal, not physically trained or a tired person, show in both directions a more or less irregular pattern. The SKG is relatively small and deviates in most cases slightly from the centre (Fig. 2–1).

With the eyes closed the pattern is somewhat more irregular. Besides frequencies between 0.5 and 2 Hz there is an indication of a frequency of about 0.2 Hz.

Otherwise healthy persons, but without labyrinths, and all persons with physically well

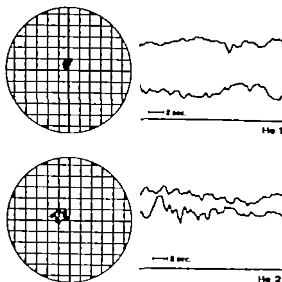


Fig. 2. Spot stabilogram (SKG) and linear stabilogram of a normal, not trained person. Upper figures: eyes open, lower figures: eyes closed.

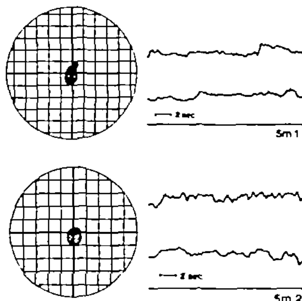


Fig. 3 Stabilograms of a person without labyrinths. Upper half: eyes open lower half: eyes closed.

trained bodies, e.g. sportsmen and dancers, have very quiet stabilograms even when they stand with their eyes closed (Fig. 3) Only the sways with relatively high frequency are present. People without labyrinths as well as well-trained people obviously need only their trained ... e motor system to stand perfectly

What happens if this proprioceptive system especially in its spinal reflexes is more or less hampered? Fig. 4 shows us the stabilograms of a patient under influence of diazepam (Valium®) This drug gives especially an inhibition in the "gamma loop" Consequently an hyporeflexibility and a lengthening of the reflex time will result. The amplitude of the curves becomes larger and the frequency by retarding, lower These curves are to be interpreted as a proof that the proprioceptive motor system is slower and plays a major part in normal cases. Perhaps the vestibular system plays a part too (see later).

Patients suffering from Menière's disease show very irregular curves (Fig. 5). *Note the* large amplitude with a low frequency of about 0.2 Hz.

In our Menière patient we saw that vestib-

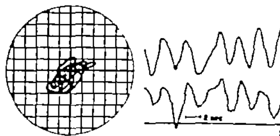


Fig 4 Stabilograms of a person under influence diazepam (eyes closed).

ular impulses are likely to manifest themselves in large sways of about 0.2 Hz. But to measure the influence of the vestibular apparatus exactly we have to measure the movements of the head as well. The vestibular apparatus reacts namely only to change in speed and as the vestibular apparatus is fixed in the head *we have to measure the movements of the* head. Our extended apparatus made it possible to do so (see Fig. 1).

In Fig. 6 the four curves of a normal, not trained person are registered. The curves of the head-stabilometry are very quiet. There is scarcely visible, a low frequency parallel with the body stabilogram especially in the left-right direction. The fast movements of the body stabilogram are absent in the head stabilogram.

We can comment to the model of Nashner et al. the human body acts like a reversed pendulum in such a way that the fast movements of the lower part of the body are absorbed by elasticity and flexibility Thus can the vestibular system protect against disturbances of higher frequencies.

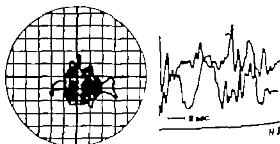


Fig 5 Stabilograms of a sufferer from Menière disease (eyes closed). Especially in the lower curve (left-right direction) a 0.2 Hz sway is manifest.

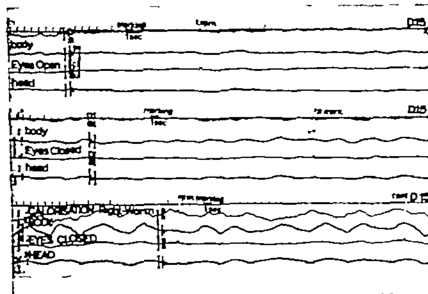


Fig. 6 Body and head stabilograms of a normal, not trained subject. Note the 0.2 Hz sway in the left-right STG in the eyes-closed position, still more *obvious in the left–right* STGs after calorization.

Unfortunately the physiological stimulus-changing in velocity cannot be shown in *posturography But we have substitutes: the* caloric stimulation which also brings the labyrinth fluids in circulation, and the galvanic stimulation. Both have disadvantages, in the caloric test only the system of the circular canals is stimulated and in the galvanic test the whole system and not only the periferal organ is stimulated. Fig. 6, lower part, shows the caloric stimulation of a normal, well-trained person. The right ear is stimulated with warm water The stimulation results in a parallel sway of a frequency of about 0.2 Hz of specially the left–right curve of body- and head-stabilogram (the amplification of both curves is not the same).

The relation between the curves, especially the left–right curves, will be the subject of our further investigations. We analysed our curves with the help of a PDP 8-computer We will not mention the full program of this data-processing here, but we will illustrate our procedures with the analysis of the above mentioned calorization of a normal person. A very pronounced sway of low amplitude is seen. The digital frequency analysis and auto-correlation show the low frequency-sway of about 0.2 Hz clearly (Fig. 7).

A stabilometric investigation of a Menière patient is shown in Fig. 8. The sway with a *frequency of 0.2 Hz is clear The body sways,* according to Nashner as a reversed pendulum. The head sways in such a way that now and then the reference point is out the picture of the camera.

The output of the head and body stabilometry in the low frequency-sway are practically the same. Therefore it is not necessary in daily practice to perform the technically difficult stabilometry of the head. We can for practical reasons limit ourselves to platform (body) stabilometry We can also limit our investigations in most cases to the left–right stabilometry as this curve gives us most information.

In this article, written with the purpose to give an insight into the possibilities of posturography for vestibular investigation we paid attention to the stabilometry (STG) But statokinesimetry measuring of the area and deviation of the statokinetic spot, gives us much information too.

DISCUSSION

In short, we can recapitulate the parameters of the posturography (statokinesimetry SKG and stabilometry STG) in this way

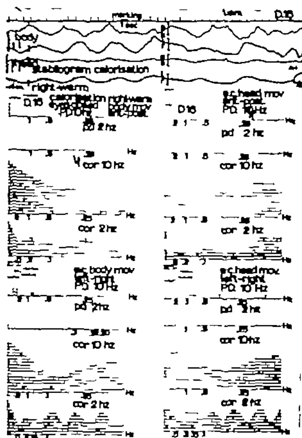


Fig. 7. Curves and computer analysis of the head and body STG after calorization (warm, right). Computer analysis. The left column is related to the body movements, the right one to those of the head. Of each row the upper half concerns Ant Post., the lower half L–R STGs. Every fourth part contains downwards: 1 a peak-time interval histogram, sample rate 10 SPS. 2 a peak-time interval histogram sample rate 2 SPS. 3 the autocorrelation curve, sample rate 10 SPS. 4 the autocorrelation curve, sample rate 2 SPS. This latter curve (last row) shows a clear "sway" in the left–right direction between 0.2 and 0.15 Hz.

Statokinesigram (SKG)

1. The area of the spot
   normal. small
   abnormal and after vestibular stimulation: larger
2. The place of the spot
   more excentric in abnormal cases
   after stimulation. (*a*) homolaterally displaced by anodal stimulation and by stimulation with cold water (*b*) *heterolaterally* displaced by cathodal stimulation and stimulation with warm water

Stabilogram (STG)

The presence of the low frequency of about 0.2 Hz is:

(*a*) absent in well trained normal people and in people without vestibular functions.
(*b*) more or less present in tired and ill-trained people.
(*c*) *manifest* in people with vestibular dysfunction and by stimulation of the vestibular system

All three parameters have proved to be highly suitable for digital computering. They give most probably also quantitative information. Our experience in this respect is however still insufficient. It is striking that the vestibular frequency of 1/5 Hz indicates a certain inertia of the system. Many authors, e.g. Walsh discern two functions of the otolith system. Firstly the perception of movements of the

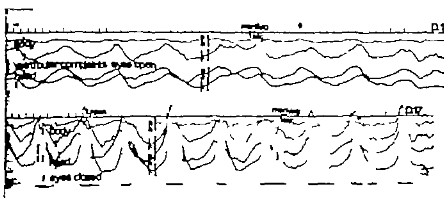


Fig. 8. Stabilograms (body and head) of a sufferer from Menière's disease. Movements of the head are bigger than those of the body

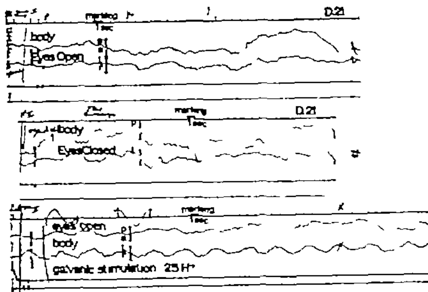


Fig. 9 Body stabilograms of a patient suffering from the vertebro-basilar syndrome. Especially after electric stimulation (0.25 Hz), vivid vestibular activity is present.

body which function is not important in our case. Secondly a function, and this function is closely related to the visual and proprioceptive system, and consequently needs a more central treatment, which is concerned with the correction of the influence of gravitation. This latter system should have an inertia of a few seconds. This would be exactly in accordance with our vestibular movements with a periodicity of about 5 seconds.

Another consideration concerns the "autofrequency" of the body. Suppose that the autofrequency of the body is in the vicinity of 0.2 Hz, one can imagine that the vestibular impulse provokes the manifestation of the 0.2 Hz sway. In this respect we have to mention that in our pilot investigation, length and weight have only small influence but that the elasticity plays a major part. The "0.2 Hz sway" of the head stabilogram is earlier

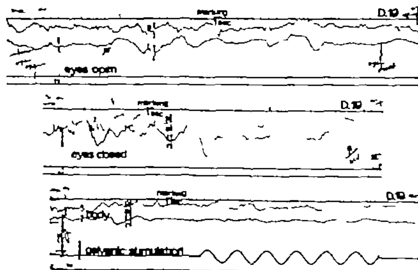


Fig. 10 Body stabilograms of a patient suffering from *an olivo-cerebellar atrophy*. No vestibular response to electric stimulation (0.25 Hz, see lower curve).

damped out in young persons than in old people.

The practice of posturography

To illustrate the clinical value of the method the records of two patients are mentioned here. The records in Fig. 9 are of a patient with a vertebro-basilar insufficiency. The galvanic stimulation delivers a magnificent autocorrelation function. The patient proves to have vivid vestibular reactions.

The second patient (Fig. 10) is suffering from olivo-cerebellar atrophy. There is no vestibular sway during periodic galvanic stimulation. The patient proves to have no vestibular function. Interesting is the result of the detection of fast frequences in this patient. By closing the eyes this patient has only the proprioceptive system to keep upright. The curves demonstrate a clear sharpening of the high frequencies (2 Hz) and during the galvanic stimulation with eyes open this extra appeal to the proprioceptis is over.

ACKNOWLEDGMENT

Njiokiktjien allowed us to publish the stabilograms of his patients. We thank Mr S. G. Mey and Mr Snel for their valuable technical help and Miss Matthys for her administrative assistance.

RÉSUMÉ

La position debout de l'homme est maintenue par le système moteur proprioceptif le système vestibulaire et les yeux. La posturographie est la registration de la force avec laquelle les pieds reposent sur une plateforme. L'analyse des courbes, une dans la direction postero-antérieure et une dans la direction gauche-droite, montrent (dans chaque courbe) deux fréquences dominantes, une fréquence base de 0,2–0,4 Hz et une fréquence plus haute de 1–6 Hz. Il est très probable que la fréquence base est associée avec le système vestibulaire. Les mouvements de la tête et du tronc sont registrés simultanément avec la posturographie. Les interrélations complexes sont discutées. Il est aussi possible de registrer l'épreuve de Romberg de cette façon.

ZUSAMMENFASSUNG

Die aufrechte Haltung des Menschen wird gewährleistet durch das motorische System, das sich selbst steuert, durch das vestibuläre Organ, das in nahem Zusammenhang dazu steht und die Augen. Posturographie ist die Registrierung der Kraft mit welcher die Fusse auf der Unterlage drücken. Die Analysen der posturographischen Kurven die die Abweichungen in der postero-antero und in der rechts-links Richtung registrieren weisen aus, dass in jeder Kurve zwei Frequenzen dominieren, eine niedrige Frequenz von ungefähr 0,2–0,4 Hertz mit relativ grossen Amplituden und eine hohere Frequenz zwischen 1–6 Hertz mit einer kleineren Amplitude. Es ist sehr wahrscheinlich, dass die niedrige Frequenz in Zusammenhang mit dem vestibulären Organ steht. In diesem Falle haben wir eine neue Methode zur Prüfung der vestibularen Funktion gefunden. Mittels eines geschlossenen Fernsehn-Kreises werden die Bewegungen von Kopf und Körper gleichzeitig mit den posturographischen Kurven geschrieben. Die Posturographie gibt uns die Möglichkeit, die Romberg-Prüfung zu registrieren.

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DISCUSSION

A Montandon. Did you try to establish some statistical correlation between the static deviation of the body and the slow phase of the head or ocular nystagmus?

R Hinchcliffe I wonder whether it is now possible to distinguish between subjects with functional ataxia and those with ataxia due to some organic lesion. For many years the clinical psychologist has used his so-called "Body Sway Test" as a personality measure, unaware of the otologist's use of the Romberg test who is equally unaware of the "Body Sway Test"

P Pialoux: Je soulignerai simplement une de nos constatations qui répond en partie aux questions de M. Montandon — les réponses aux excitations vestibulaires apparaissaient dans l'ordre suivant proprioceptives — inclinaison de l'ensemble du corp — nystagmus.

de Wit (Reply) to Mr *Montandon.* Indeed we registered many times stabilometric and nystagmographic curves simultaneously (projection of slide). We did not pay attention to a correlation between the slow phase of the nystagmus and the velocity of the / Hz-sway Perhaps Mr Montandon's suggestion can be worked out in this respect.

To Mr *Hinchcliffe:* A "functional" i.e. not organically provoked ataxia can sometimes be traced by stabilometry The hysteric ataxia has an unnatural" curve. Although the curve does not show the proportion of proprioceptive or vestibular disturbances the patient has the tendency to fall down now and then. Extremely nervous or anxious patients have a vivid pattern of proprioceptive activity while the curve becomes more quiet after medication with a sedativum.

To Mr *Pialoux:* We too have the impression that vestibular disturbances manifest themselves sooner in the stabigram than in the nystagmogram.

Acta Otolaryng 73 112–120 1972

THE COCHLEAR AQUEDUCT AND THE PRESSURE OF CEREBROSPINAL AND ENDOLABYRINTHINE FLUIDS

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Abstract. Cerebrospinal fluid pressure changes are reflected in perilymph via the cochlear aqueduct and are passed on to the endolymph, probably via Reissner's membrane. The hypothesis that the endolymph pressure is transmitted by the cerebrospinal fluid via the endolymphatic sac and duct appears to be refuted by our results. In the normal labyrinth the perilymphatic and endolymphatic pressure are equal within the accuracy of our measurements. The effective cross section of the cochlear aqueduct together with the expansion properties of the round window membrane may serve as a protective mechanism against sudden changes in the cerebrospinal fluid. A pressure gradient between endolymph and perilymph will have to stay below 2 cm of water a value at which rupture is to be expected. Therefore damage of the sensory cells due to pressure in case of an endolymphatic hypertension is very improbable, provided the perilymphatic pressure is not affected which is likely when the cochlear aqueduct is open. The expansion of the endolymphatic compartment in a relatively short time appears to go as far as four times it s original volume after which rupture occurs.

The value of the cochlear aqueduct for the endolabyrinthine fluid pressure becomes obvious by comparing the results of the pressure measurement of perilymph and endolymph before and after blocking this aqueduct. As the cochlear aqueduct goes from a niche at the round window towards the cranial cavity our interest in the cerebrospinal fluid pressure is evident. Our greatest problem was finding a suitable method to measure the pressure of perilymph and especially of the endolymph. Like Weille et al. (1958, 1961) and Martinez (1969) we tried to measure the endolymphatic and perilymphatic pressure via the bony cochlear wall in the guinea pig. After one frustrating year in which some 300 guinea pigs were sacrificed, we realized no worthwhile results could be achieved in this manner This experience along with a scrutiny of the publications of Weille et al. (1958 1961) and Martinez (1969) made us doubt the efficacy of the method and caused us to search for a better procedure for pressure measurement. Weille et al (1958) had tried also the route of the round window membrane in order to measure the perilymphatic pressure in 100 guinea pigs; they reportedly encountered severe leakage problems along the microcannula, being the front part of the measuring system In the 25 experiments we performed, we found leakage without fail. To overcome leakage, a special technique was developed which we later perfected to the point that even the endolymphatic pressure could be measured, namely via the basilar membrane without mixing perilymph and endolymph along the microcannula In finding a method for endolabyrinthine pressure measurement, we became extremely interested in the pressure gradient between perilymph and endolymph. Weille et al. (1958, 1961) and Martinez (1969) concluded from their 5 year period of experimental work,

For elaborate details refer to "On the Pressure of the Endolymphatic, the Perilymphatic, and the Cerebrospinal Fluid, With Data on the Endolymphatic Membrane" by B. I. J Beentjes. North-Holland Publishing Company Amsterdam and London, 1970 (thesis).

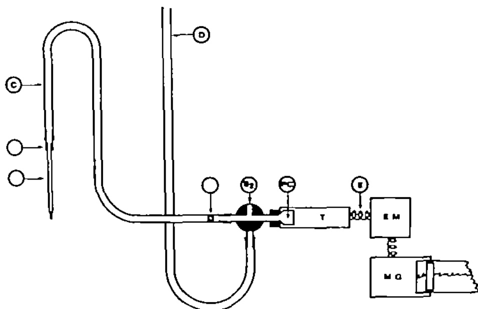


Fig. 1. Schematic diagram of measuring system. A, microcannula (Pyrex); B, connective piece (polythene); C, measuring cannula (polythene); D, calibration cannula (polythene); E, connecting cable; EM, electromanometer; MG, mingograf; PC, pressure chamber; PT, pressure transducer; S_1, three-way stopcock, allowing connection between *PC* and *D*, *PC* and *C* and *PC*, *D* and *C*. X, xylol.

that in a live guinea pig, the perilymphatic pressure exceeds the endolymphatic. We measured the pressure of perilymph and endolymph sequentially and in one manoeuvre with the same probe and detecting system. Thus we avoided the problem of constructing two measuring systems displaying identical characteristics under the various actual operating conditions necessary to detect small differences in pressure values.

Another group of experiments were performed to see how much the pressure difference between the endolymph and perilymph could be before breaking of presumably Reissner's membrane.

MATERIAL AND METHOD

For our pressure measurements we employed a pressure transducer, an electromanometer and a multi-channel fluid-jet recorder, all manufactured by the Swedish Elema-Schönander Company. Pressure in the endolabyrinthine fluid was transmitted to the pressure chamber of the pressure transducer via fluid present in the intermediate tubing system, which consisted of a Pyrex microcannula, a polythene connective piece, a polythene cannula, and a three way stopcock (Fig. 1). Simultaneous measurement of the d.c. potential at the extreme end of the microcannula enabled us to establish the entrance of the microcannula tip into the endolymphatic space through the basilar membrane. Therefore a chloridised silver wire entered the intermediate tubing system in between the connective piece and polythene cannula. From this entrance site the wire channelled down to midway to the microcannula from whereon saline or Ringer solution took over the electrical connection to the tip end of the microcannula. A small amount of xylol (in Fig. 1 indicated by "X") flanked by conducting fluids in the polythene cannula near the three way stopcock served as an electrical insulator.

The inside diameter of the Pyrex microcannula was 1.2 mm with a wall thickness of 0.3 mm, while the inside diameters of the

microcannula tip ranged from 20 to 90 μm with a wall thickness of 10 to 40 μm.

Our new approach towards the endolaby rinthine fluids for pressure measurements no longer favoured the guinea pig with his pig mented stria vascularis as the experimental animal. Therefore our further measurements were carried out on cats.

Fig 2 shows a microcannula and the basilar membrane viewed through both the secondary tympanic membrane and the intermediate peri lymph after opening the posterior room of the cat s bulla Note the few capillary vessels in the round window membrane.

The microcannula tip was pre-treated by the application of a contact adhesive[1] at the tip upto the very rim which effectively coped with the problem of leakage The contact adhesive provides a self settling packing. A certain portion of the compressible adhesive remains at the tip end of the microcannula after passing the round window membrane which serves as the seal when the basilar membrane is pierced. The site of perforation of the basilar membrane was chosen where it bordered the spiral ligament Fig. 3 demonstrates the micro- after retraction following a success- measurement. We performed experiments in vitro to prove the effectiveness of the contact adhesive as a preventative against leakage. Before and often also after each measure ment, we performed calibration of the measuring system.

For the cerebrospinal fluid pressure measurement we performed a lumbar laminectomy to expose the dural sac. Via an opening made in the dura, we inserted an intravenous cat heter into the cerebrospinal space Leakage along the catheter was prevented at this stage by applying Eastman 910 adhesive all around the site of insertion Further fixation of the catheter was assured by filling the wound around the catheter with miniature pieces of gauze, after which the external wound was closed. The catheter was connected with a pressure transducer and electromanomet similar to that used for the endolabyrinthi pressure measurements.

[1] "Soelfix" diluted with tolual, Soelfix is manufactured by Cetabever Beverwijk, Holland.



Fig 2. The basilar membrane viewed through b the secondary tympanic membrane and the int mediate perilymph. A microcannula directed towa the basilar membrane is shown also.

In the cases we wanted to have a block cochlear aqueduct, a hole was drilled med caudal to the round window approximately ha way to the cochlear aqueduct The direction which we drilled was more or less perpend cular to this aqueduct. When this duct w reached, it was obstructed with dental cemen We studied the place and direction of th cochlear aqueduct on a number of skulls usin an inserted horse hair as an indicator (Fig. 4

RESULTS

The results of our endolabyrinthine pressur measurements will be given first.

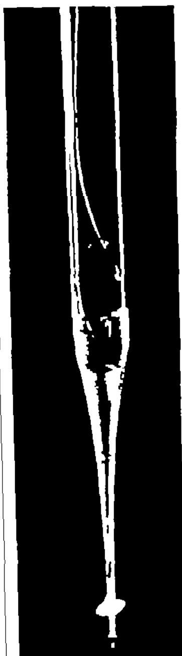


Fig. 3. The microcannula after retraction following a successful measurement. The upper and the lower ring of contact adhesive are the results of encounter with the round window and basilar membrane, respectively.

Fig. 4. The skull of a cat in which a hole (*small arrow*) was drilled perpendicular to the cochlear aqueduct. A horsehair (*large arrow*) was inserted for the purpose of checking.

Just after piercing the round window membrane with the tip of the microcannula, a pressure build-up to equilibrium occurred. The time for reaching equilibrium is of the order

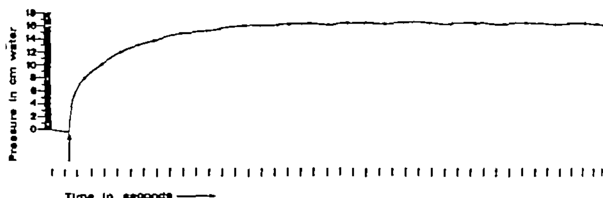


Fig 5 Recording of the pressure when the microcannula tip pierces the secondary tympanic membrane at the time indicated by an arrow Note the pressure build-up to equilibrium. The abscissa gives a time scale in seconds, the ordinate a pressure scale in cm of water

of 1 min. Differences in build-up rate are influenced by the bore of the microcannula tip (Fig. 5) In perilymph and endolymph pressure lines fluctuations are demonstrable reflecting the respiration (Fig 6) In several cases superimposed shorter waves reflecting the heartbeat were visible also (Fig 6) The appearance of these heartbeat fluctuations bears a relation to the inside diameter of the microcannula tip

When the microcannula tip was advanced into the endolabyrinthine space in 25 μm steps by means of a micromanipulator these steps were noticeable by a temporary pressure increase of short duration (Fig. 7)

Apart from the influences of respiration, heartbeat and insertion of the microcannula tip equally demonstrable in both the perilymphatic and endolymphatic pressure measurements, the pressure line displays still another type of fluctuation (Fig. 6) The order of magnitude of this fluctuation is a few (up to 3) mm of water in an interval of variable duration sometimes as short as 10 sec. (Corresponding irregular waves in the pressure line of the cerebrospinal fluid were detectable also.)

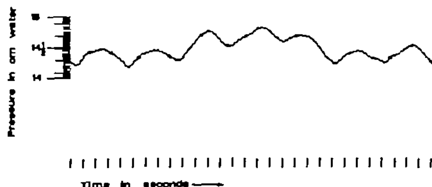


Fig 6 A fluctuation in the perilymphatic pressure line with superimposed influences of respiration and heartbeat. These pressure variations were equally demonstrable in the endolymphatic pressure curve and matched the perilymphatic curve of the same animal.



Fig 7 A plot of pressure and potential versus time. The middle line indicates the pressure (as read on the left ordinate) in advancing the microcannula tip by 25 μm increments at a time: on the left in the perilymphatic compartment, on the right in the endolymphatic compartment, in the intermediate region the microcannula touches and pierces the basilar membrane. (*a*) end of heartbeat fluctuation. (*b*) end of respiration fluctuation. (*c*) reoccurrence of respiration fluctuation. (*d*) reoccurrence of heartbeat fluctuation. The bottom line indicates the d.c. potential as read on the ordinate on the right. The marks in downward direction, shown on all registration lines, indicate the beginning of each additional penetration over 25 μm into the endolabyrinthine space and are artificial. The topline functions as a time indicator

In our sequential perilymphatic and endolymphatic pressure measurements we were highly selective so that only 12 of some 80 measurements qualified. These 12 experiments provided perilymphatic pressures during 1 min before piercing the basilar membrane and endolymphatic pressures during 1 min there after We averaged these two sets of pressures, and the differences (ΔP = perilymphatic pressure minus endolymphatic pressure) are listed in Table I. The perilymphatic pressure proved to be equal to the endolymphatic pressure within the margin of uncertainty of the measurement (1 to 2 mm of water cf Fig 6)

The absolute endolabyrinthine pressure varied in these 12 measurements from 11 4 to 18 0 cm of water with an average value of 14.5 cm. The endocochlear potential varied from 72 to 108 mV with an average value of 96 mV (Table I) Fig. 7 is typical for a sequential perilymphatic and endolymphatic pressure measurement in the cat

The cerebrospinal fluid showed similar fluctuations reflecting the respiration but far more pronounced (Fig. 8) Actually these fluctuations in the endolabyrinthine pressure line turned out to be an attenuated reflection of fluctuations in the cerebrospinal fluid this we have concluded from the observation that the endolabyrinthine pressure line looses the breathing variations when closing off the aqueduct. In order to check how much of this attenuation might have been caused by the Pyrex microcannula with a tip of 80 μm outside diameter we connected this small-tipped microcannula to the much wider catheter through which the cerebrospinal fluid pressure variations have been measured. The microcannula did have a definite smoothing effect on the curve, but the cochlear aqueduct appeared to have an even stronger smoothing effect which is understandable considering its small effective cross section In addition, the possibility of expansion of the endolabyrinthine space by the round window membrane may contribute to this smoothing effect. Applica-

tion of abdominal pressure increases the cerebrospinal fluid pressure up to 2 or 3 times and is coupled with an equal rise in perilymphatic pressure However the perilymphatic pressure reaches its top slowly upon an abrupt rise in cerebrospinal fluid pressure When abdominal pressure is no longer applied, the abrupt fall in cerebrospinal fluid pressure is accompanied by a slower but equal fall in perilymphatic pressure. This is equally applicable to perilymph and endolymph. We applied an over pressure to the cerebrospinal fluid also and apart from a time lag, this overpressure was followed exactly in perilymph and endolymph. We did this in 5 cats with overpressures of approximately 5 cm of water We found in those 5 cases the cerebrospinal fluid pressure approximately 1 cm of water higher than the endolabyrinthine fluid pressures. This might be an artefact because of the usage of two measuring systems.

In 9 cats we have blocked the cochlear aqueduct The endolabyrinthine fluid pressures were measured 1 hour after blocking in 4 of these cats and these pressures were measured several weeks after blocking in the other 5 cats. - did not see differences in our measurement s between these two groups. Normally the endolabyrinthine pressure amounts to ap-

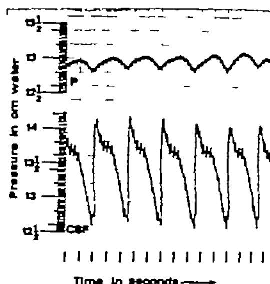


Fig 8 Plot of pressure variations in the cerebrosp fluid (*CSF*) due to breathing and the reflec thereof in the perilymphatic pressure (*P*). Note attenuation occurring in the upper curve. A car inspection also reveals that this curve lags bel the cerebrospinal fluid curve.

Table I. *Absolute pressure values for perilymph differences in pressure between perilymph and endolymph and values for the endocochlear d.c potential*

Experimental cat number	Perilymphatic pressure (cm water)	ΔP in mm of water (see text)	Endolymphatic d.c. potential (mV)
39*	11 4	0.8	96
37*	12.6	0.0	90
35*	13.0	1.2	84
31	13 6	0.0	87
33*	13.8	−0.4	96
23	14.2	−2.0	90
42*	14.4	0.0	87
17	14.8	0.0	108
25	14.8	2.0	72
19	15.2	−0.4	102
41	17.2	1.0	84
34*	18.0	−1.2	96

proximately 14 cm of water After blocki this pressure was considerably lower rang from 0 0 to 9.2 cm of water with an avera of 4 cm More important is that norma after depressurization of the perilymph atmospheric value, the endolabyrinthine pr sure recovers completely within minutes, l when we blocked the cochlear aqueduct, endolabyrinthine pressure remained n atmospheric value. The breathing fluctuati were absent in all "blocked" animals. T blocking operation had no effect on the end lymphatic potential. We detect no differen in pressure between the perilymph and end lymph.

For determination of the maximum pr sure difference between perilymph and end lymph until break down of presumably Rei ner s membrane we removed partially t round window membrane This made the pe lymphatic pressure atmospheric. The end lymphatic pressure then proved to be al atmospheric We determined how much ov pressure with respect to atmosphere could

applied now to the intact endolymphatic compartment before rupture occurred and for how long. Such an overpressure causes an influx of some fluid into the endolymphatic space. The inflowing fluid had been colored with trypan blue to serve as a marker in case of rupture. The endolymphatic compartment appeared to remain intact with a pressure head in the size of 1 cm of water for a duration of 15 min or possibly longer. As deduced from a sharp increase in the influx rate and as checked by the appearance of trypan blue in the perilymph, the endolymphatic compartment ruptured for certain with a pressure head of 2 cm of water. The total amount of fluid which could enter the endolymphatic compartment before rupture was approximately 10 mm³ in the seven cases investigated.

RÉSUMÉ

Nous avons enregistré chez des sujets normaux le nystagmus postrotatoire après des rotations horaires et antihoraires avec stimulus d'intensités différentes. Nous avons évalué les composantes horizontales et verticales du nystagmus dans les histogrammes. Cette méthode d'évaluation permet d'obtenir plus d'information sur l'état fonctionnel du système vestibulaire, en particulier sur le système vestibulaire efférente.

ZUSAMMENFASSUNG

Das Verhältnis zwischen dem Cerebrospinalflüssigkeitsdruck und dem Druck der Endolymphe und Perilymphe bei der Katze wurde vor und nach Sperrung des cochleären Aquäduktus studiert. Mit diesen Experimenten wurde die Wert des cochleären Aquäduktus fur den endolabyrinthären Druck klar. Unter gewissen Umständen fanden relativ hohe Druckänderungen sowohl in der Perilymphe wie in der Endolymphe statt. Andere Experimente zeigten, dass es nur kleine, wenn überhaupt welche, Druckänderungen zwischen der Perilymphe und Endolymphe geben kann

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DISCUSSION

O. Sala: Did you perform any experiments with drugs. Your results are very interesting also from a clinical point of view. I can modify the endolabyrinthine pressure by means of drugs which decrease the pressure of the cerebrospinal fluid. I use these drugs in the therapy of patients suffering from Menière's disease with recovery of vertigo and often with improvement of the hearing.

S. K. Bosher: I have had the privilege to see Mr Beentjes perform one of his experiments and can testify to his remarkable skill in this difficult field. There are two comments I wish to make about the conclusions he has derived from the studies presented today. Firstly, I think there is an alternative to the simple hydraulic process which he has suggested might account for the pressure equilibration. In our own work we have been able to demonstrate that water moves relatively freely across the cochlear duct membranes in response to osmotic forces and consequently a similar process could account for the equilibration Mr Beentjes has observed. Morphological investigation should help to differentiate which of these processes was predominant and I wonder, therefore, whether he has had the opportunity of examining his material histologically? Secondly, although the findings presented reveal little evidence of a compensatory action of the endolymphatic sac with respect to the cochlear fluids, could it not be of more importance for the vestibular labyrinth?

S. Iurato: Did you observe individual differences in the dimensions of the cochlear aqueduct in your cats? Did you make any histological control about this point? The reason for the question is that during stapedectomies in a few subjects an abnormal outflow of perilymph from the oval window was observed. This means that in these subjects the cochlear aqueduct was not obstructed by connective tissue and it was still open as during fetal life. I am wondering if these individual differences are also present in cats?

J. Tonndorf: Comment (1) I am glad that Mr Beentjes finally laid to rest the Wellie et al. notion that endolymphatic pressure (not equal to) perilymphatic pressure. (2) Explanation for poor low-frequency response of micro-cannulae lies in the nature

of things: they are high-pass filters. (3) In own experiments on bone conduction (1966) we found that when the occlusion of the aqueduct was successful the pressure on opening the cochlea was zero. Question: (In line of Mr Bosher's question). What was the histological condition of the organ of Corti after occlusion of the aqueduct?

B I J Beentjes (Reply) to Mr *Sala*. We did not attempt to alter the cerebrospinal fluid pressure by drugs with the purpose of influencing the endolabyrinthine fluid pressure. If any fluid can pass through the cochlear aqueduct (which is certainly the situation in the cat) I believe that every change in the pressure of the cerebrospinal fluid will be followed by the same pressure change not only in the perilymph but also in the endolymph. Therefore I do not see how such drugs will help in cases of Menière's disease. I can see how a difference in osmotic pressure between perilymph and endolymph can cause a distension of the endolymphatic compartment. I would be more interested in drugs that might influence the osmotic pressure in perilymph or in endolymph of a hydropic labyrinth.

To Mr *Bosher* 1) In our experiments for determination of the maximum pressure difference between perilymph and endolymph until breakdown, we increased the endolymphatic pressure in steps and applied each overpressure mostly as long as inflow of fluid into the endolymphatic compartment apparent. The total duration of overpressure va from 8 to 32 minutes but in all cases the total amo of fluid which could enter the endolymphatic comp ment before rupture was approximately 10 mm³ water can move more or less freely through the t cell layer thin membrane it must occur rather slo We did not make histological examinations of cochlear duct after the just mentioned experime It would have been to our advantage to have done 2) The only thing we concluded from our experim is that the pressure in the cerebrospinal fluid is passed to the endolymph in the cochlear duct the endolymphatic sac and duct. Our experiments not give any further information about the en lymphatic sac.

To Mr *Iurato* After each experiment we remo the round window membrane and we sometimes the impression that there was a quicker perilym flow than usual. We regret the fact that we did make histological control in such cases. We get idea about the possibilities of the free passage thro the cochlear aqueduct in the cat by the experime of Schuknecht and Seifi. They proved that chick erythrocytes could pass through this duct.

To Mr *Tonndorf* We did not perform histologi examinations on the organ of Corti after occlusi of the cochlear aqueduct.

Acta Otolaryng 73 121–125 1972

NEW POSSIBILITIES OF ANALYSIS IN ELECTRONYSTAGMOGRAPHY

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Abstract. The authors have introduced vectonystagmography for the computer analysis of electronystagmograms. *The φ angle determining* the direction of the rapid eye movements and the φ angle between the first and the second, and between the first and the third channels have been measured. The variables have been defined. A programme has been prepared where the output data give information on each individual nystagmic movement and on total values. A detailed description of data collection and programming is given, plus examples of three-channel vectonystagmographic recording.

For an exact analysis of the electronystagmogram it is necessary to have *exact information* of the eye movement. A single derivation is not sufficient.

If in the raw channel there is a horizontal nystagmus, all the data will be exact. But if the nystagmus is oblique the amplitude will appear smaller and so also will the velocity of the slow component. If the nystagmus is vertical, there will be no registration on the horizontal derivation. *With three-channel electronystagmography we avoid such errors.*

Two electrodes are situated temporally and the third on the forehead all electrodes are active so that they give three channels. If the eye movements are conjugated, they work like a moving dipole so that from the registration in three channels the real eye movement can be reconstructed in the same way in which we determine the electric axis of the heart by Eintoven's triangle (Fig. 1)

In horizontal nystagmus, the sum of the upward movements of both eyes will be registered in the first channel, if the direction *of the nystagmus is to the right* (Fig 2)

In the second channel the spikes will also face upwards, but they will be only half as large In the third channel the spikes will be negative, i.e. pointing downwards, but of the same amplitude as in the second channel (Fig. 3)

If the nystagmus is oblique, so that it is perpendicular to the third channel, then the amplitude in the first channel will be smaller than the real movement of the eye, while in the third channel there will be no registration at all (Figs. 4 5)

If the nystagmus is perpendicular to the second channel, there will be a complete absence of registration (Fig. 6)

If the nystagmus is vertical, in the second and the third channels the direction of the nystagmus is the same, while in the first channel there is no registration at all (Figs. 7 8)

In the conventional, single derivation, the *vertical nystagmus can easily be overlooked, though this nystagmus is an indicator of central* damage

If the nystagmus is almost but not completely vertical, showing a slight inclination towards the left or right, there will also be a weak registration in the first channel. It is possible to make a mistake in conventional derivation. the vertical nystagmus is taken for the horizontal, which can lead to an erroneous diagnosis (Fig 9).

Summarizing these few examples, we come to the conclusion that the advantage of three channelled nystagmography lies in the fact *that it records the real direction of the nystag*

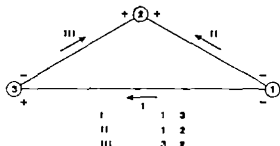


Fig 1 Electrodes 1 2, 3 Channels: I (1-3), II (1-2), III (3-2). + record upwards; − record downwards.

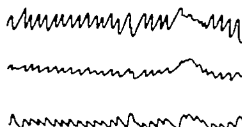


Fig 3 Horizontal nystagmus to the left in the channels.

mus, excluding the possibility of the vertical nystagmus being overlooked or mistaken for a horizontal one, or the amplitude and velocity of the slow component of the oblique nystag mus being recorded as smaller values. If the oblique nystagmus appears only in some but not all caloric tests, the three-channelled nystagmography excludes possible error in the relation of irritability of the right and left labyrinth, or mistakes in the preponderance of the direction of nystagmus towards one side

There is finally a question of the possibility of determining the exact direction of the nys- ius, and not only of its derivations. This,) is important and must not remain uncontrolled.

In order to obtain exact values of the ele ments of nystagmus by three-channelled elec tronystagmography (exact direction, ampli tude velocity of the slow component) it is necessary to work out the data of the three channels. In order to achieve this for every nystagmic movement in the course of the whole vestibulometric examination (spon taneous, fixating positional, optokinetic, calo-

ric nystagmus) it was necessary to mal programme for computer analysis (Fig. 1(

The direction of eye movement is d mined by the angle ψ in which zero degr in the horizontal plane on the left, and angle increases counter-clockwise The a φ is the angle between the first and the sec and first and third channels. Its average v is 20 to 25 degrees

The amplitude of the registration in the channel is $U_{D1}+U_{L1}$ in the second cha U_{L2}, and in the third channel U_{D3}.

The real direction of the right eye m ment is calculated by the following formu

$$tg\psi D = -\frac{2U3}{U1 \sin\varphi} + ctg\varphi$$

and the real direction of the left eye movem by the formula

$$tg\psi L = \frac{2U2}{U1 \sin\varphi} - ctg\varphi$$

From these data, the real amplitude U_L the left and U_D of the right eye can be work out.

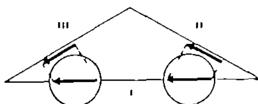


Fig. 2. The projection of eye movements in channels I, II and III.

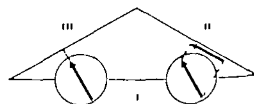


Fig 4 Eye movements perpendicular to the t channel.

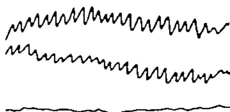


Fig 5 Oblique nystagmus perpendicular to the third channel.

We start from the assumption that the amplitude of both eyes is equal,

$U_D = U_L$

and that the direction of both eyes is the same

$\varphi D = \varphi L$

From the perforated cards the machine selects the data about the angle and then about the amplitude in each channel (first, second and third. U_1 U_2, U_3)

If the data are not complete, the programme will complete them and if they are complete, the programme continues to determine the principal parameters.

The programme marks the direction of the nystagmus: right (registration upwards) as plus, left (registration downwards) as minus the fast and slow components of the nystagmus are determined the tangents of the angle for both eyes are determined from these data the real direction of eye movement of the right and left eyes is worked out the real amplitude for both eyes is calculated having considered all this, in further procedure the real value of the



Fig 6 Oblique nystagmus perpendicular to the second channel.

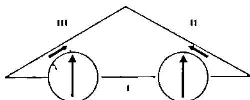


Fig 7 Vertical eye movements.

velocity of the slow component for both eyes is stated.

All data are sent through the printer and if the procedure is not completed, the programme returns the whole material to the phase after registration, starting with the phase of determination of the principal parameters.

After this, the printer receives total values the duration of the nystagmus, the entire amplitude, the sum of the nystagmic movements

The *first* condition for a good analysis of electronystagmographic data is an exact registration of the eye movements.

The *second* condition is that all elements of the nystagmus and all relations are given in numerical values When all data for the analysis and diagnostic synthesis can be expressed in figures, they can be used for any computation and in any procedure (computer communication, teleprinter)

In this way we ensure the best and safest transmission, processing and comparison of data, with minimal danger of the number of data being diminished or deformed in the process of transmission in space and time.

Expressing the elements of the nystagmus from its direction to the dysrhythmia in figures, the computer analysis of data already gives us the following information



Fig 8. Vertical spontaneous nystagmus.

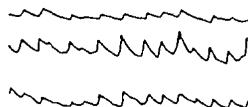


Fig 9 Vertical nystagmus slightly inclined to the right.

1 the exact direction of the nystagmus and thus the exact values of all elements of the nystagmic movements
2. *amplitude*
3 *duration*
4 *velocity of the fast component*
5 *velocity of the slow component* as the most important single measure of the intensity of the nystagmus
6 *analysis of the slow component* changes of its velocity acceleration at the beginning, deceleration before changing the direction (sharp or rounded peaks on the recorder)
7 *the boundary between the conjugated and dissociated nystagmus*
8 *the threshold of appearance of the fast component*
9 *changes of this threshold*
10 analysis of *the dysrhythmic nystagmus* in four groups
(*a*) dysrhythmia frequency and amplitude, since they are reciprocally proportional by the same velocity of the slow component ($s + a\ f$)
(*b*) dysrhythmia of the velocity of the slow component (s)

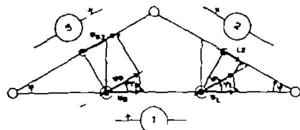


Fig 10 The main variables for computer analysis of the nystagmus.

(*c*) $a + b$
(*d*) intermittent nystagmus
11 analysis of the elements of the *spontaneous nystagmus*
12. analysis of the positional nystagmus, its duration analysis of all elements of the nystagmus and the relation of the nystagmus to the position of the head and body
13 analysis of the elements and their relation in the fixating nystagmus:
(*a*) in primary zero position, and
(*b*) in secondary positions: dextroversion, sinistroversion, supraversion and infraversion of 20 degrees
14 analysis of elements of *the optokinetic nystagmus*
15 latency increase and decrease of the provoked *caloric nystagmus* in all elements
16 the entire provoked nystagmic response in individual elements, but primarily the entire amplitude or better *the sum of average velocity of the slow component in all individual seconds* of nystagmic response, as the most important entire measure
17 *the relation of the right and left labyrinth in the caloric test*
18 *relation of the direction of the nystagmus to the right and to the left in the caloric test*
19 *secondary nystagmus* in caloric test and its relation to the primary nystagmus in any of the nystagmic elements
20 difference of strength of the elements of the nystagmus in fixation and non-fixation in this way the intensity of suppression of the nystagmus during fixation (failure of fixation-suppression, FFS) is expressed. It is obtained by a simple subtraction of the velocity of the slow component (s) in non-fixation (NF) from the velocity in fixation (F) $s\ NF - s\ F = FFS$
21 changes of intensity of suppression (FFS)

We believe that work on the realization of such a programme of data analysis enhances extension of the procedure: diagnostic synthesis of the analysed data We believe it to be

necessary in order to take advantage of at least a part of the message concerning the organism which is incorporated in the nystagmus.

RÉSUMÉ

Les auteurs ont élaboré la vectonystagmographie pour l'analyse électronystagmographique au moyen d'un calculateur électronique. L'angle ψ qui détermine la direction du mouvement de l'oeil, et l'angle φ qui existe entre le premier et le deuxième canal et entre le premier et le troisième canal sont calculés. La définition des variables est élaborée. On a établi le programme pour calculer les données de chaque secousse nystagmique et du total des mesures. Les auteurs décrivent le programme et présentent des exemples d'enregistrement vectonystagmographique sur trois canaux.

ZUSAMMENFASSUNG

Für die Analyse des Elektronystagmogrammes mit Hilfe des Computers, erfanden die Autoren die „Vectonystagmographie". Es wurde die Ecke ψ errechnet welche die Richtung der Bewegung des Auges bestimmt und die φ Ecke welche zwischen dem ersten und zweiten, und zwischen dem ersten und dritten Kanal besteht. Die Variablen werden genau definiert. Es wurde ein Programm ausgearbeitet, das beim Ausgang die Daten für die einzelne nystagmische Zuckung und auch die gesammt Werte ergiebt. Die Autoren stellen den gesammte Programm der Daten Verarbeitung dar. Sie erbringen die Beispiele der dreikanaligen vectonystagmographischen Aufzeichnung.

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DISCUSSION

C. R. Pfaltz. In which way are the nystagmograms analyzed? Is each nystagmogram scrutinized individually by a doctor or a technician or by an integrator? How can differences of responses within the 3 recordings, which are due to technical factors (change of resistance of electrodes—changes of the periocular electrical field etc.) be distinguished from those originating from vestibular effects

G. de Wit. Mr Padovan goes out from the preassessment that the movements of the two eyes are always and in all circumstances quite the same. I doubt if it is always true. We measured in our laboratory the nystagmus of every eye separately and simultaneously. Many times there were differences in direction and in intensity between the two eyes.

R. Hinchcliffe: This method could solve some of the problems concerning the large variability even in normal subjects of the results relating to the magnitude of caloric induced nystagmus as recorded with the conventional horizontal placement of the electrodes. It is possible that some of the variability is due to the non-horizontal direction of nystagmus in certain subjects and the variability of the slopes. Could Mr Padovan let us hear the dispersion of measures revealed in this manner compared with that of those recorded with the conventional horizontal placement of electrodes.

I Padovan (Replies): Electronystagmography has not yet a generally accepted standardization but only conventions which cause difficulties. We have 8 years of experience using all results of Montandon, Jongkees and the others. In our audiovestibular laboratory we have 3 doctors and 3 technicians. Every patient has 1 hour of examination followed by 20 minutes of computations on the university computer centre. All the parameters are indicated on our special forms. We use first the manual method in measuring and then the data are sent to the computer. We had 5 000 cases of which 2 500 were submitted to conventional electronystagmography and 2 500 to the 3 derivating electronystagmography so that we can compare these two methods. We have seen much fewer mistakes in diagnosis with the method of 3 derivation electronystagmography

Acta Otolaryng 73 126–129 1972

THE TIME HISTOGRAM AS A WAY TO EVALUATE THE TRANSITION FUNCTION OF THE VESTIBULAR SYSTEM

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We are studying the vestibular transition function in normal subjects, specifically the temporal course of postrotatory nystagmus I with different speeds at the stop.

For stimulation we use the Tönnies rotation chair with the subject seated and his horizontal semicircular canals in the plane of rotation. The eyes are closed and the room is in darkness. Alternatively clockwise and counter clockwise rotations are performed, with growing accelerations and velocities in the stop, until we have a total of ten rotations for each subject The accelerations used are 0.5 1 3 6 d 8 sec² reaching angular speeds of 30, 60 120 180 sec, respectively The sudden stop is carried out after maintaining the constant speed for two minutes, and between one rotation and the next, four minutes rest are permitted. It is a technique of stimulation similar to the cupulometry by Jongkees.

The postrotatory nystagmus is recorded electronystagmographically after visual calibration in the cross of Maddox. The record is obtained by means of periocular electrodes in binocular derivation. Both the horizontal and the vertical components of the ocular movements are registered. An a.c amplifier is used with a time constant of 1 sec and a chosen graph paper speed of 5 mm sec.

Once the electronystagmographic records are obtained, the quantitative evaluation of the postrotatory nystagmus is carried out by measuring the following variables, all of them corresponding to the fast phase (*a*) amplitude and moment of production of each beat. This enables the drawing of the time histogram and the deduction of the other parameters (*b*) total amplitude (*c*) duration of nystagmus; (*d*) number of beats (*e*) frequency (*f*) mean amplitude (*g*) typical velocity that is equal to the product of the mean amplitude by the mean frequency

With the preceding data are elaborated the time histograms that express graphically the course of the nystagmic response. The time histogram is obtained drawing a chart and plotting on the abscissa the time of production of each beat from the beginning of the nystagmus. The amplitude of each beat is plotted on the ordinate The results of the clockwise rotation are represented as positive values and those of the counterclockwise rotation as negative ones (Fig. 1) Two charts of this type are obtained for each final velocity one belonging to the horizontal component and the other to the vertical one.

Finally the relation between the stimulus strength and each one of the previously mentioned parameters is represented. (The typical velocity seems to be the most characteristic one) (Fig 2.)

RESULTS

A. *Formal analysis*

The time-histogram seems to be a very clear method to represent the temporal course of

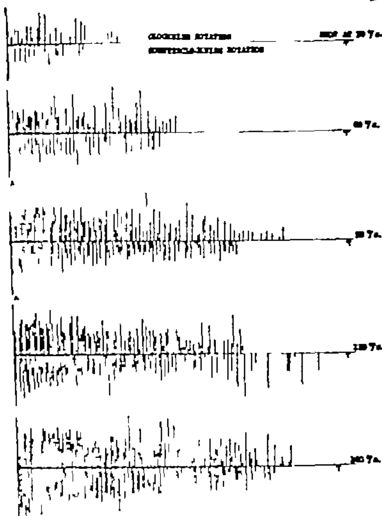


Fig. 1. Time-histograms from a normal subject with different speeds at the stop. *Ordinate:* Amplitude of each nystagmic beat in mm. 2 mm = 1 degree. Positive values: response to clockwise rotation. Negative values: response to counterclockwise rotation. *Abscissa:* Time in mm. 5 mm = 1 sec.

the postrotatory nystagmus. The form and magnitude of the transition function can be examined at a glance and it is possible to compare rapidly the response to the rotation in opposite directions.

In the temporal course of the nystagmic response a sinusoidal modulation of amplitude is observed. This wave-like rhythm is very similar to the modulation of the alpha-rhythm that appears in the EEG of young persons. It would be interesting, from our point of view to study conjunctly the characteristics of both phenomena in the same individual, since their frequencies should probably coincide due to their common origin coming from the interaction of the reticular formation, the diffuse thalamo-cortical projection system and the Hernández Peon system.

In several of the histograms studied, all of them belonging to healthy subjects, we have observed periods of silence or interruptions in the nystagmic response that cannot, therefore be considered as pathological signs (Fig. 3).

B. *Statistical evaluation*

The statistical analysis will lead to the discovery of the mean values and standard devia

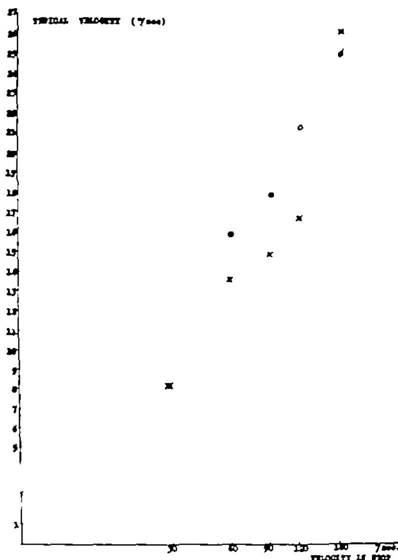


Fig 2. Relation between the angular velocity at the moment of the stop (*abscissa*) and the typical velocity of the subsequent nystagmus (*ordinate*). O clockwise rotation; counterclockwise rotation.

tions in normal subjects in order to decide which one of all the parameters studied is most characteristic of the present phenomenon

Although we do not yet have the results of the statistical study we can suggest that a remarkable difference exists between the responses to rotations in opposite directions for the same velocity and individual. Symmetrical responses, without being unusual, are not the rule. In the same manner the relation between increase of stimulus and increase of response is quite variable from one subject to another being replaced in the corresponding graphs to lines of different slopes.

This wide variation of responses in normal subjects leads one to think that in the transition function of the vestibular system, a fundamental role is played by the arousal level of the reticular formation at the moment of the sudden stop Therefore a multitude of factors modify the response attention and interest of the subject in the test, fatigue, mental activity —all of them factors that are variable from one moment to another

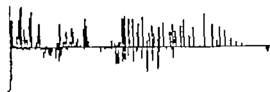


Fig 3. Time-histogram from a normal subject. Notice the asymmetry between the clockwise and counter clockwise responses and the pauses existing in the nystagmus.

With repetition of the stimulus a habituation seems to exist, not in the peripheral receptor but in the reticular systems that medicate the motor response. Such habituation, however is not uniform for the different subjects and may be counterbalanced by several factors that maintain a high level tone of the activating reticular system.

We consider that the transition function constitutes a very elaborate response of the whole nervous system to the vestibular stimulus. And that this response provides a great amount of information in reference to the functioning of the Central Nervous System and of the vestibular system in particular.

RÉSUMÉ

Nous avons enregistré chez des sujets normaux le nystagmus postrotatoire après des rotations horaires et antihoraires avec stimulus d'intensités différentes. Nous avons évalué les composantes horizontales et verticales du nystagmus dans les histogrammes. Cette méthode d'évaluation permet d'obtenir plus d'information sur l'état fonctionnel du système vestibulaire, en particulier sur le système vestibulaire efférente.

ZUSAMMENFASSUNG

Bei normalgesunden Menschen wurde der postrotatorische Nystagmus nach verschieden starker Drehreizung elektronystagmographisch registriert. Beide Komponenten, vertikal und horizontal, der ausgelösten Augenreaktionen in beiden Drehrichtungen und nach Reizungen zunehmender Intensität wurden nach der Methode des Zeithystogrames ausgewertet. Diese Methode scheint mehr als die übliche Auskunft über den funktionellen Zustand des Vestibulären Systems zu geben, besonders über das efferente Vestibularissystem.

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DISCUSSION

J. Groen: I understand that Mr Bartual's experiments dealt with cupulometry but I could not gather whether his slides represented histograms of eye velocity (left right) or eye movements (amplitude) as a function of time or of another parameter. Would he be so kind as to explain his recordings.

J. Tonndorf: I understand under "histogram" something different. What Mr Bartual has shown is a simple time amplitude relation, *but* not a histogram.

J. Bartual (Reply) to Mr *Groen*. In einem doppelten Koordinatensystem wurden auf der Abszisse die Auftrittszeiten jedes einzelnen Nystagmusschlages, vom Stop der Drehung ab gezählt, aufgetragen. Auf der Ordinate wurde die Amplitude des entsprechenden Nystagmusschlages übertragen. Als positive Werte wurden die Ergebnisse der Rechtsdrehung, als negative die der Linksdrehung übertragen.

To Mr *Tonndorf:* Unsere Begriffe über das Hystogramm scheinen nicht identisch zu sein.

Acta Otolaryng 73 130–140 1972

COCHLEAR ADAPTATION CENTRAL INFLUENCES

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Abstract Cochlear adaptation can be divided into a peripheral and a central form; the latter influences the cochlea via the crossed and uncrossed olivary bundle. The adaptation can be studied by recording the AP generated in cochlea or cochlear nucleus by presenting clicks to the ear of the test animal. In order to differentiate between peripheral and central influence on cochlear adaptation, the cochlea is isolated from its central part of the auditory tract by ablation techniques. As long as the cochlea is connected to its central pathways the relationship between click intensity and AP magnitude is linear from threshold up till 30–40 dB above threshold, whence it levels off asymptotically If however the cochlea is isolated, this relationship becomes linear After cutting of the crossed olivo-cochlear bundle an influence is only found at threshold levels; the threshold is lowered thus cochlear sensitivity is increased and the signal noise ratio is diminished. Apparently cochlea is not under any adaptational influence er of central or of peripheral origin, in the sity range from just above threshold to 30–40 dB.

The description by Rasmussen (1942, 1946 1953 1960) of the direct and crossed olivo-cochlear bundle gave a new impetus to the study of the efferent neural influence on the cochlea. Iurato (1964) demonstrated that the crossed cochlear efferents in the rat only reached the outer hair-cells for the uncrossed efferent fibres, the site of action is suggested to be the afferent fibres in a prae-synaptic way (Smith & Sjöstrand 1961) and the inner hair cell (Iurato 1964), although there might be some difference between the rodent and the cat The function of the crossed olivo-cochlear bundle is mostly studied by electrical stimula tion of the bundle *in the floor of the fourth* ventricle (Galambos, 1956)

This work was supported in part by a grant from the Netherlands Organization for the Advancement of Pure Research (Z.W.O.)

It was found that there was a certain depre sion of the total action potential (AP) th depression depended upon current and repet tion rate of the used electrical stimulation. Th effect did not exceed 15 dB Desmedt (196 repeated this kind of experiment. His resul were nearly the same, except that the depre sion now reached a value of about 20–25 dB To elicit more information about the singl neuron depression Fex (1962) measured th change of activity in the single auditory nerv fibre he found that the resting activity an the sound-induced activity were reduced. A typical finding in all measurements so fa mentioned was the relatively long latency a the start and end of this kind of adaptation so that it is questionable whether such *adaptation is due to the function of the olivo-* cochlear bundle or to a slow activity in highe order neurons of the auditory tract as wa suggested by Whitfield (1967)

The resting activity of the olivo-cochlea tract as measured by Fex (1962) showed a constant discharge of a very regular characte rated about 10 pulses per second This resting activity could be of great importance con cerning the sensitivity level of signal/noise ratio of the peripheral organ controlled by a constant inhibitory factor Experiments in volving the influence of the contralateral cochlea via the olivo-cochlear bundle were per *formed by Galambos (1956)* and Hilger et al (1966) who found a response from the ipsi lateral cochlea when stimulating the contra lateral cochlea acoustically It could not be demonstrated from which part in the auditory

tract the responses arose. Still there is a possibility that higher parts of the contralateral auditory tract have been measured. Pfalz (1969) even denied the function of the olivocochlear bundle and stated that electrical stimulation could give rise to an unphysiological procedure.

More recently the function of the olivocochlear bundle was studied by Nieder & Nieder (1970) who found that this bundle is involved in perception of pitch and in the discrimination between complex auditory stimuli. It was found, after stimulation of the crossed bundle, that the signal-to-noise ratio of auditory nerve activity improved, according to the measurement of Dewson (1967 1968), who also found a blocking of the low-level threshold fibres. The adaptation effect on the cochlea, divided in a crossed and an uncrossed one, acts in a different way: the crossed part could be more involved in threshold adaptation.

We do not yet have a satisfactory answer to the question whether the adaptation, when it is not purely peripheral (Kupperman, 1971), arises from the central influences of higher centres via the crossed bundle of Rasmussen, or from the uncrossed part, or from both.

In our experiments we tried to separate sequentially the peripheral end-organ from its higher neural connections by ablation techniques. The influence upon the cochlea of higher neural centres in the auditory tract can be measured by first removing the nucleus cochlearis. Influence via the bundle of Rasmussen can be studied by carefully removing the contralateral cochlea and its higher centres. The total neural activity (AP) of the cochlea was used because only then can the threshold of the total neural answer of the cochlea be measured

METHODS

The main problems in measuring in the central nervous system are the topographic localization of the electrode inside the brainstem and the constant physiological behaviour of the test animal.

For this purpose a new test animal from Mongolia was introduced in our laboratory: a kind of mouse named Meriones (Fig. 1) (Schober & Brauer 1967) in which the brainstem can be explored easily in a translabyrinthine way. The nucleus cochlearis could easily be located at the entry of the auditory nerve into the brainstem. Into the cochlear nucleus a micro-electrode or a thin silver wire (100 μm diam.) was inserted by which responses to clicks presented to the cochlea were picked up. The activity of the different parts of the auditory tract were measured by opening the brain between the horizontal canal and the crus commune (Fig. 2)

Now it was possible to measure activity of the auditory nerve, the nucleus cochlearis and higher centres in the auditory tract. The latency made an easy separation possible between the firing of the auditory nerve and the activity of the nucleus cochlearis. The meriones was anaesthetised with urethane which gave a reliable and long-lasting anaesthesia. A normal air passage was possible without the need of a tracheostomy. To avoid any influence from the middle earmuscles, both were cut during the following experiments.

The electrical input was a pulse with a duration of 100 μsec and a rise-and-fall time of 100 nsec

The repetition rate, intensity and interval time of the successive clicks could be varied. The bio-electrical output of the cochlea was measured at the round window or through a hole in the scala tympani of the first turn. After a noise free amplification of × 10 000 the signal was fed to a CAT 1000 computer and after summation recorded on a X Y writer

RESULTS

The measurements of the AP

In order to obtain comparable AP measurements under different circumstances the parameters intensity repetition rate and interval-



Fig. 1 The test anim Meriones in a typical right position.

time could be varied. A toneburst of sequential spikes with an interval of 5 msec and a total duration of about 50 msec appeared to be an ideal acoustical stimulus to start with.

An input–output function when only the intensity was varied gave us most of the information we need Its intensity was increased by steps of 5 dB At threshold intensity level, 10 000 samples were summated threshold level was considered to be reached when the re sponse became just visible on the oscilloscope screen. In this way magnitude and latency of the first AP were measured peripheral adaptation showed itself clearly in the ensuing cording.

The magnitude of the AP was measured the area of the N1 that was found under baseline The latency was measured as time-delay between the onset of the electri stimulus and the negative dip N1 of the AP

Although it is better to use the cochl microphonics (CM) to N1 latency this pro dure is likely to fail at low intensities becau of the disappearance of the CM The inp output curve of the normal functioning coc lea is portrayed in Fig 3



Fig. 2 The mastoid and middle ear cavity of Meriones surgically opened. The semicircular canals, the cochlea and the translabyrinthine approach to the brainstem between the anterior semicircular canal and the crus commune are portrayed. The eardrum covers a part of the cochlea in the intact conduction system.

Influence of the total efferent system on the cochlear neural output

The efferent connections of the cochlea run through the auditory nerve. In order to investigate the influence of the higher centres on the cochlea, sectioning of the auditory nerve is most favourable, but in most cases the blood supply to the cochlea is also damaged. In our experiments another solution is found by suction of the cochlear nucleus after its electrical localization. Only when most of the environment of the nucleus was removed, were certain characteristic changes in the appearance of the AP visible

It was clearly seen that primarily the total area of the AP was enlarged (Fig. 4) When the cochlea is totally isolated from the central part, yet the blood supply is still intact, a maximum area of the AP is reached a delay of 15 minutes is allowed to ensure a stable

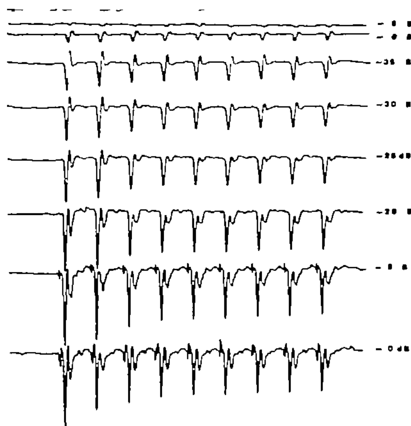


Fig. 3 APs measured at the round window: the intensity is increased with steps of 5 dB. The cochlear adaptation is clearly demonstrated at the higher intensity ranges. The interval between successive clicks is 5 msec. The dB scale refers to the threshold −5 dB.

…d supply to the cochlea then an input–… curve is made the intensity of the …ntered click being increased in steps of 5 dB. It has been found that from threshold intensities upwards the input–output curve before and after sectioning does not alter till an intensity of about 30 dB above threshold is reached. In the intensity range 30–65 dB above threshold the input–output relation is different before and after sectioning. Before sectioning, the curve tends to level off asymptotically to a maximum, whereas the input–output curve after sectioning continues its straight course. Also, the shape of the AP altered after sectioning. It was found that the amplitude input–output curve stayed constant but that the base of the triangle was lengthened up to 5 msec. In this way the total area of the AP was enlarged (Fig 5). Before and after sectioning of the connections between the nucleus cochlearis and cochlea no influence on the typical peripheral cochlear adaptation could be observed (Fig 4).

Another typical finding was that there was a decrease of the threshold of the AP of about 4 to 6 dB (further measurements will be found in the next section). These experiments were repeated after 1 hour and 3 hours in some cases but no alterations in output or shape of the AP were found.

Measurements of the contralateral activity of the cochlea

To be informed about the functional cochleo-cochlear interrelationship the ipsilateral cochlea was resected carefully in such a way that the modiolus was kept intact. By means of an electrically sharpened tungsten wire of 100 μm the habenula perforata was penetrated so that a connection was made with the underlying auditory nerve; this procedure was repeated for the different turns of the cochlea. It was found

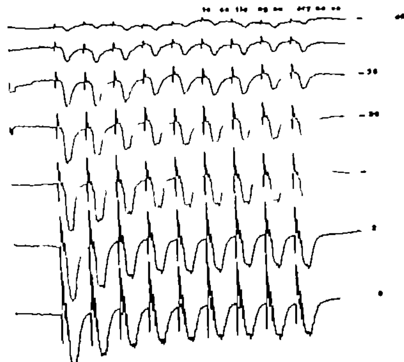


Fig. 4. APs measured in the same way as in Fig. 3 after sectioning of the auditory nerve, by removing the nucleus cochlearis at the same side. Note remarkable difference in area of the normal and affected AP at the higher intensity ranges. Note also that the CM is enlarged in amplitude.

that after summing up × 10 000 a bio-electrical potential arose with a latency of about 3–4 msec which was independent of the electrode placement, having only a slight difference in latency probably due to the conduction time in the auditory nerve. The gross appearance of this bio-electrical potential measured at the auditory nerve consisted of at least two positive going potentials with a latency of 3 msec and 4.5 msec and with amplitudes having a constant ratio of approx. 2 : 1. To obtain more information about the different potentials, the AP of the contralateral cochlea and the neural activity in the contralateral nucleus cochlearis were measured as described earlier (Fig. 6).

It was found that the AP arose with a latency of 1.5 msec and that most of the activity measured at the ipsilateral isolated auditory nerve was generated in the nucleus because of the same latency of approx. 3 msec. The other positive potential with a latency of 4.5 msec must have been generated in a higher centre in the contralateral auditory tract because of its longer latency. This latency indicated that at least two extra synapses were involved. Most of the activity measured at the isolated auditory nerve is generated in the contralateral nucleus cochlearis and has nothing to do with the efferent system of the ipsilateral cochlea whatsoever.

Ablation of the contralateral cochlea nucleus cochlearis and crossing fibres

To obtain information about the influence of the contralateral cochlea and its higher centres, the different parts of the contralateral auditory tract were destroyed. The influence on the input–output function of the ipsilateral AP was measured.

First of all both cochleae were explored. The APs were measured and compared when both APs were equal as far as gross appearance, input–output function and threshold were concerned, the contralateral cochlea was carefully eliminated. The ipsilateral cochlear input–output curve was measured again. There was no difference in the threshold, even when the contralateral nucleus cochlearis was re-

moved *in toto* Only when a greater part of the environment of the nucleus was removed and the midline sectioned was the threshold of the AP lowered, by 4–6 dB Still (Fig. 7) the input–output curve for the higher intensity range stayed unchanged the APs too had the same appearance before and after damaging of the contralateral auditory tract.

DISCUSSION

Adaptational processes of the cochlea can be divided roughly into processes inside the cochlea as described in a previous paper (Kupperman 1971) and into a central feed-back system whose centripetal influence on the cochlea is most accessible for measurement. This efferent system (Rasmussen) can have a crossed and an uncrossed character

Both systems could influence the cochlea in a specific way Another inhibitory system is the effect from higher on lower auditory centres, as for example the influence of the olivary complex on the nucleus cochlearis these complex influences have not been considered in this work We tried only to find a ...s influence of the central parts of the ...y pathway on the total action potential ...P) was used, since it represented the firing of a great number of haircells when the cochlea was acoustically suitably activated.

In our findings it was obvious that the influence on the cochlea, whether due to a crossed or an uncrossed system, could only be measured by carefully observing the area of the AP This area, as it represented the activity of the total number of active hair-cells, was significantly influenced when the connections between the cochlea and its higher centres was interrupted after sectioning, the input–output relation (between AP area and intensity) became linear whereas it previously levelled off at approx. 30 dB in an intact system The form of the AP also changed and the enlargement of the base of the triangle could be due to a desynchronizing of the firing of the hair-cells combined with an increase of the number of

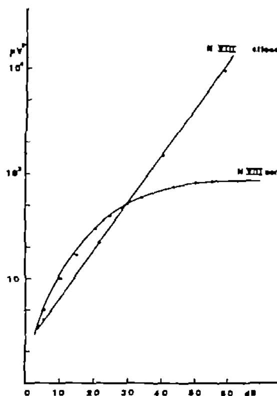


Fig 5 Input–output curve for the normal A compared with input–output curve for the altered A after removal of the nucleus cochlearis. Note t typical linear character of the function of the alter AP above threshold.

active hair-cells This way of cutting the aud tory nerve had of course some disadvantage it was impossible to locate the exact plac where the efferent influence on the cochle was generated Neither could it be determine whether a crossed system or an uncrossed or was involved The fact that the input–outpu function of the isolated cochlea became line was of interest.

It was found that below an intensity o about 30 dB no difference between the isolate and normal cochlea existed, so in a certai intensity range there is no central inhibitor influence.

One problem arose it was not possible t ascertain so precisely the delay in the adaptin system as was possible in experiments in whic the bundle of Rasmussen was stimulated.

To divide the efferent influence in a crosse

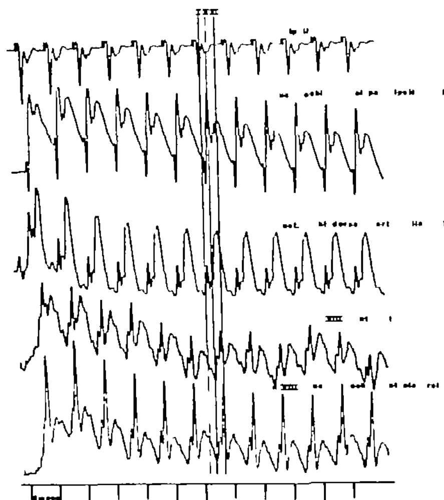


Fig. 6 The bio-electrical potential measured at different stations in the auditory tract. It is demonstrated that the latency between the different potentials is a realistic method to trace locus of origin. Note the polarity change to positive when the measurement concerns a central activity. Number I indicates the time of the negative N_1 of the AP of the intact cochlea. Number II indicates the N that corresponds with the positive activity of the auditory nerve entering the nucleus. III corresponds with the over all activity of the nucleus cochlearis at the ipsilateral side. Note that this activity corresponds to the activity measured at the isolated auditory nerve at the contralateral side. The latency differences of 1.5 msec are in agreement with the synaptical transfer time. The used intensity was 50 dB above threshold.

and uncrossed part, the contralateral cochlea was destroyed there was no influence on the ispilateral cochlea. This was in agreement with the measurements of others, e.g. Pfalz (1969), who found that there can be a limiting influence on the ipsilateral cochlea only when a certain intensity range is exceeded this adapting influence on the AP can be found only when there is also a cross-over hearing through the skull which can even reach a value of 30 dB depending on the form and structure of the test animal's skull. This kind of measurement has to be avoided. Thus, in between certain limits the cochlea works quite stable without further influence from the central parts of the auditory tract. It is possible to measure the activity of the contralateral ear by careful exploration of the auditory nerve it is of great importance whether the bio-electrical potentials found in the isolated audi-

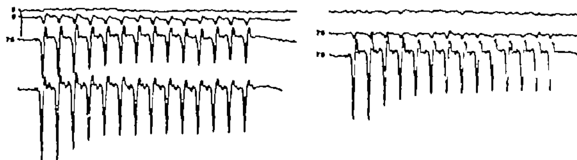


Fig 7 The APs after sectioning of the bundle of Rasmussen. It is clearly demonstrated (left) that the threshold has shifted to a more sensitive value by 5 dB compared with the intact (right) side. Note that at −70 dB the magnitude of the successive A before and after sectioning is the same. The numb refer to distal settings and indicate only 5 dB ste

tory nerve are really transferred by means of the bundle of Rasmussen or whether these are measurements of activity in the other contra lateral centres of the auditory tract. By measuring the different stations in the contralateral part of the auditory tract it was found that almost all the activity of the ipsilateral nerve when stimulating the contralateral cochlea with clicks, was due to activity of the nucleus cochlearis at the contralateral side only a small part was due to olivary complex activity This ans that an inhibitory activity from the l side via the bundle of Rasmussen at least doubtful for the intensity range ove 30 dB We can only conclude from our measurements that almost all the efferent influence on the cochlea is generated in the centres at the ipsilateral side probably the uncrossed efferent fibres of the olivary complex inhibit a great deal of the outer hair cells.

Of course we were very interested in the function of the crossed olivo-cochlear fibres. For this purpose not only is the ablation of the contralateral cochlea of interest but also the sectioning of the bundle of Rasmussen in the floor of the fourth ventricle The results of these experiments were a lowering of the threshold of the AP with a constant value of about 5 dB It can be concluded that when there is a function of the crossed olivo-cochlear tract this is not an inhibitory one working over a wide intensity range but only an i hibitory one shifting the threshold of t cochlea slightly

This small threshold change works as improvement on the signal-to-noise ratio. Fro our experiments it is not clear whether th function is due only to resting activity of th auditory tract or to neural activity dur cochlear stimulation at higher intensity leve

This concept is in agreement with the fin ing of Fex (1965) who found a certain resti activity by measuring with electrodes in th olivo-cochlear tract, and with the measur ments of Nieder who suggested that an im provement of the signal to-noise ratio may b an important function of the crossed oliv cochlear tract.

These types of ablation technique as use in our experiments always have the possibili of damaging more of the central nervo system than exclusively destroying parts of th auditory tract however when respiration an heart rhythm stay constant between certai limits, the metabolic conditions of the brai stem cannot be so greatly influenced.

Conclusions can be drawn as follows.

1 The uncrossed olivo-cochlear bundle in fluences the cochlea above an intensity of 30 30 dB in such a way that the action potentia (AP) levels off asymptotically When the audi tory nerve is cut the input–output function o the AP becomes linear

2. The crossed olivo-cochlear bundle is not so active at higher intensity levels as was presumed only an influence on the sensitivity threshold of the cochlea was found in such a way that the signal/noise ratio was increased.

3 After cutting of the auditory nerve the typical cochlear adaptation was not influenced.

4 The adaptational influences on the cochlea, whether of central or peripheral origin, do not work in the intensity range from just above threshold up to 30–40 dB

Further investigations have to be performed in which, with pharmacological techniques, the synaptic transfers in the different ganglion cells are blocked a check on the foregoing experiments is then possible

RESUMÉ

L influence de la partie centrale du trajet auditif sur le rapport input-output de la cochlée est mesurée à base du potentiel d'action (P.A.) total, comme une indication de l'output nerveux cochléaire. Un autre type de cobaye dont le noyau cochléaire permet une exploration translabyrinthaire facile est introduit. La fonction du potentiel d'action est mesurée avant et après la destruction du plusieurs parties du trajet auditif. J'ai pu constater qu'il y a une influence specifique du noyau cochléaire sur la fonction du potentiel d'action telle qu après sectionnement du nerf auditif le niveau du potentiel d action excède la valeur normale lorsqu on augmente l'intensité des stimulations au-dessus d'une certaine valeur Je n ai pas du démontrer l'influence du faisceau olivo-cochléaire en stimulant l'oreille contralatérale.

ZUSAMMENFASSUNG

Cochleare Adaptation kann unterteilt werden in eine periphere und eine zentrale Form, diese letzte beeinflusst die Cochlea mittels die gekreuzten und nicht gekreuzten Oliven-Bahn. Die Adaptation kann untersucht werden durch Registrierung des Summenaktions-Potentials (AP) in der Cochlea des Versuchtieres durch Klicks aufgeweckt. Um den Unterschied zwischen periphere und zentrale Einflusse zu ermöglichen wird die Cochlea isoliert von ihren zentralen Bahnen durch Ablationtechniken. So lange die Cochlea verbunden ist mit ihren zentralen Bahnen ist die Beziehung zwischen Intensität und AP linear von der Schwelle ab bis 30–40 dB uber der Schwelle, wonach die Kurve umbiegt und zu einem Maximum kommt. Wenn die Cochlea dagegen isoliert ist wird diese Beziehung linear im ganzen Gebiet Wenn die gekreuzte Oliven-Bahn durchtrennt wird kann nur ein Einfluss festgestellt werden in der Schwelle Nähe. De Schwelle wird erniedrigt, die cochleare Empfindlichkeit wird gesteigert und das Verhältnis der Nutz und Störsignalen verschlechtert. Wahrscheinlich ist die Cochlea nicht adaptiert weder von zentraler oder peripherer Ursprung im Intensitätsbereich von der Schwelle aufwärts bis 30–40 dB.

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E.N T Clinic
University of Utrecht
Utrecht
The Netherlands

DISCUSSION

F Escher In destroyed labyrinth after fracture or section of the auditory nerve patients often complain about extremely disagreeable *acouphens*. Did you observe spontaneous action potentials from the proximal end of the auditory nerve?

M Portmann. Ayant travaillé beaucoup les fibres efferentes je voudrai faire deux commentaires: 1) En ou 56 j'avai recherché sur le cobaye la réponse olaterale à un clic avant et après destruction de 'lle contralaterale La réponse N3 disparaissait destruction contra-laterale. Etait-ce un artefact ou N3 pourrait il être du au fonctionnement des fibres efferentes homolaterales 2) Demain je presenterai des déformations de l'electrocochleogramme humain affectee de troubles retrolabyrinthiques identiques aux images presentées par Mr Kupperman? Notamment un peu positif précédent l'élargissement de N1 a-t-il une application physiopathologique de ces modifications?

J Tonndorf I should like to congratulate Mr Kupperman for the choice of a proper animal on which to conduct his experiment. This is very often a ver crucial point in an experiment.

Concerning the change of the input/output cur that originally has a very limited linear range an after destruction of the cochlear nucleus this rang increased considerably I could give what I belie to be a good explanation if Mr Kupperman had don the opposite experiment, i.e., if he had destroyed part of the peripheral organ, but I am at a loss as to th explanation of his observation. I would like to as him to speculate a little on the underlying mechanism

U P Fisch. I have been very interested to see tha Mr Kupperman found a broadening of the AP fol lowing section of the cochlear nerve. We observe the same phenomenon following section of the XII nerve in cats and we interpreted this finding as a los of synchronization in the response of the single nerv fibre. In view of the changes in the hearing functio following section of the olivo-cochlear bundle i humans (Fisch, 1970)—i.e. loss of the ability t localize sounds in space—it would be interesting t speculate if the loss of synchronization of the AI could be the explanation for it.

R. Kupperman (Reply) to Mr *Escher* After ablation of the cochlea no activity is found in the auditory nerve but there is an increase of the spontaneous activity of the nucleus cochlearis at the same side.

To Mr *Portmann.* The finding that the N3 of the AP will disappear after ablation of the contra-lateral cochlea can be due to the fact that there is a cross-hearing at high intensities generating activity in the contra-lateral nucleus cochlearis. After ablation of centre this N3 will disappear The finding of a positive peak before the enlarged AP can be due to the enlargement of the CM as a consequence of the disturbance of the efferent system.

To Mr *Tonndorf* The change of the input/output function after ablation of the nucleus cochlearis at the same side must be due to the failure of the efferent system that influences the total number of firing haircells and probably the synchronizing of the firing of the haircells.

To Mr *Fisch* As I said before, not only is the number of retine haircells involved when the efferent system to the cochlea is cut, but there is also an effect on the synchronizing of the firing haircells which is demonstrated in the enlargement of the base of the AP

Acta Otolaryng 73 141–147 1972

ÉTUDE AUDIOMÉTRIQUE ET ÉLECTRO-NYSTAGMOGRAPHIQUE CHEZ L'HOMME NORMAL — EN PLONGÉE TRÈS PROFONDE

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Marseille France

Abstract Les fonctions auditives et vestibulaires ont été examinées chez quatre plongeurs, préalablement sélectionnés et testés. L'étude de l'audition a montré, aux différentes pressions, une élévation des seuils en voie aérienne, la conduction osseuse restant inchangée. L'examen vestibulaire avec E.N.G. est resté normal. Les difficultés de compréhension liées aux perturbations phonétiques, ont été éliminées grâce à l'utilisation d'un vocodeur (Bathyphone I. B. M.).

L'étude physiologique de l'homme lancé dans l'aventure subaquatique se poursuit depuis quelques années, et des résultats ont déjà été rapportés, dans les domaines de la mécanique ventilatoire et de la fonction cérébrale notamment

Le comportement de l'appareil auditif et vestibulaire, très peu étudié jusqu'à présent, a pu être testé grâce à une collaboration déjà ancienne avec le Professeur Chouteau, Directeur Scientifique au Centre d'Etudes Marines Avancées, où se sont déroulées des expériences de plongée fictive en saturation à grande profondeur Les hommes, plongeurs émérites et entraînés, ont séjourné à diverses profondeurs, 250 m pour l'expérience dite Saturation I, 400 m pour Saturation II.

Il s'agissait de plongées en oxygène hélium (la pi O_2 étant maintenue à 400 m) avec paliers longs, lors de la remontée, autorisant diverses explorations.

Notre but, lors de ces premières plongées en saturation à grande profondeur était double d'une part déterminer de façon la plus précise possible la présence ou non d'altérations auditives sous l'effet de l'élévation de pression et des mélanges gazeux utilisés, d'autre part rechercher à l'aide de l'enregistrement E.N G avant et après stimulation, l'existence éventuelle d'une dysréflexie vestibulaire

ÉTUDE AUDIOMÉTRIQUE

Celle-ci a été menée avec le plus de rigueur possible pendant l'enregistrement des audiogrammes le silence le plus complet possible était réalisé autour et dans le caisson. Tous les systèmes de ventilation et de régénération à l'intérieur et à l'extérieur de la chambre hyperbare étaient arrêtés. Le niveau du bruit ambiant dans ces conditions est demeuré au tour de 35 dbs, ce qui correspond à peu près à celui relevé dans une cabine d'audiométrie clinique.

La technique a été celle de l'audiométrie automatique de Békésy à son pulsé, à cause de ses avantages remarquable stabilité des courbes chez le même sujet, même si elles sont prises à des intervalles de temps éloignés, établissement d'un diagramme enregistré de façon continue sur la totalité du champ auditif testé, permettant des comparaisons et un calcul statistique.

Il a été utilisé un audiomètre AP 6 Peters, soigneusement étalonné, avec des écouteurs type TDH 39 (10 ohms), et le vibrateur mastoïdien Radio Ear 8.70 A.

Seuls l'écouteur et le bouton de commande sont dans le caisson, l'appareil étant situé à l'extérieur

Il n'a pas été possible de réaliser une audiométrie vocale car le temps consacré à ces ex

périences était rigoureusement limité par l'arrêt de la ventilation qui faisait monter la pression de CO_2 dans le caisson dans d importantes proportions.

Mais des tests psychologiques par ailleurs régulièrement répétés aux différentes profondeurs sont restés normaux, ce qui prouve l absence de toute narcose chez ces sujets.

Chaque sujet avait subi un audiogramme automatique en C.O et V.A avant l'expérience et en a subi un autre plusieurs jours après, qui s est avéré identique au premier Il avait été entrainé à se servir de l'appareil.

Un audiogramme a été enregistré dans les deux expériences d'abord dans le caisson mais à l air et à la pression atmosphérique puis aux divers paliers suivants (en O_2 He évidemment)

Sat I	Sat II
11 ATa	31 ATa
16 ATa	36 ATa
21 ATa	41 ATa
26 ATa	

Cette pression était maintenue constante pendant toute la durée de l'épreuve. On enregistrait successivement V.A. et C.O pour D et pour l O G pour le premier puis le deuxième plongeur

La durée totale de l'examen a été de 1 heure 30 chaque fois à la même heure de 15 heures à 16 heures 30

RÉSULTATS

Tous les tracés sont interprétables du fait de l excellente coopération des plongeurs testés. La seule difficulté rencontrée selon eux est la gêne importante représentée par la perception intense des bruits organiques (respiration, déglutition, etc)

Ils sont présentés sous forme de diagrammes qui montrent

— les tracés audiométriques réels, obtenus en audiométrie automatique.

— les tracés du seuil audio-liminaire théorique, obtenu en faisant la moyenne de l'écart des pointes.

Les tracés objectivent

— *en voie aérienne* l'existence d'une élévation des seuils liminaires pour les fréquences basses et moyennes, jusqu à la fréquence 1 500 Hz environ, et dont la valeur maxima varie de 15 à 25 dB (Fig. 1) Les 4 plongeurs ont présenté ce phénomène. Par contre, seuls les tracés des plongeurs de Sat II objectivent une élévation liminaire également au niveau des fréquences aiguës (entre 3 000 et 6 000 Hz), mais de valeur sensiblement comparable (15 à 25 dB) (Fig. 2)

On constate que les seuils théoriques liminaires ne varient pas de façon rigoureuse en fonction de l élévation de la pression.

— *en conduction osseuse* les tracés ne montrent pas de variations sensibles de la courbe osseuse en fonction de la pression. Il semblerait même que la perception soit légèrement meilleure aux fortes pressions, mais la dispersion apparaît importante notamment au niveau des fréquences aiguës.

Le contrôle avant et après ne montre pas de changement.

COMMENTAIRES

Discussion des résultats

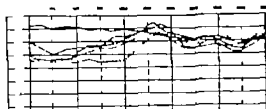
La lecture et l'interprétation de ces résultats imposent la notion, en ambiance hyperbare d O_2–He, d une baisse de l'audition par voie aérienne, prédominant au niveau des fréquences graves, et d'une conduction par voie osseuse peu perturbée.

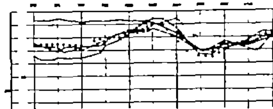
Ces constatations rejoignent celles de J Adolfson et E. Fluur (Aerospace Medicine Feb 67) et nos constatations personnelles lors de l'expérience Choutaqua (1965)

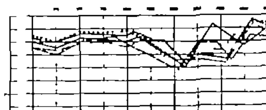
Il faut cependant souligner trois points

— d'une part, les examens audiométriques tonaux et vocaux d Adolfson ont été réalisés en air à 4–7 et 11 aTa, l'importance des perturbations de la discrimination auditive en audiométrie vocale laissant supposer non pas une perte de capacité auditive, mais des troubles centraux liés à l'élévation de la PPN_2.

— d autre part, les conditions expérimentales en matière d'audiométrie nécessitent de







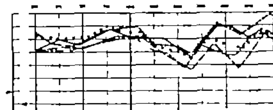
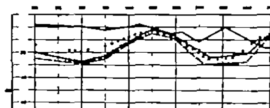
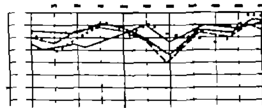


Fig. 1. Audiométrie tonale automatique, saturation I. Seuils liminaires théoriques. Sujet Gauret. —— 1 ATA, ---- 11 ATA, ——— 16 ATA, —— 21 ATA, 26 ATA.







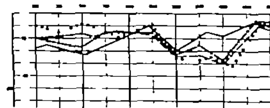


Fig. 2. Audiométrie tonale automatique saturation II. Seuils liminaires théoriques. Sujet Le Pechon. —— 1 ATA --- 31 ATA, -.-— 36 ATA, 41 ATA.

soins particuliers, notamment en ce qui concerne le réglage et le contrôle des appareils ainsi que le bruit ambiant qui doit être totalement supprimé. Pour cette raison, nos enregistrements précédents (Choutaqua, 1965), de même que des résultats obtenus par certains auteurs tel que Summit (1970), qui reconnaît un bruit ambiant de 60 à 65 dbs ne peuvent être interprétés qu'avec beaucoup de prudence.

— enfin la nécessité d'avoir dans des con-

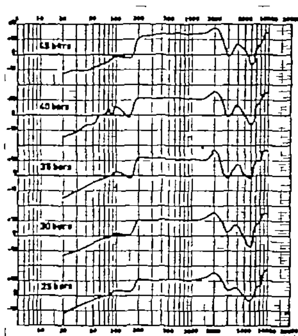


Fig 3 Différence (ΔN) entre les niveaux de pression acoustique délivrés par l'écouteur soumis à la pression *P* (niveau N) et à la pression atmosphérique (niveau N₀). ΔN = N − N₀

ditions identiques, des examens comparatifs de chacun des sujets, autorisant une étude statistique des résultats obtenus c est dans ce but que s avons choisi l audiométrie automatique Békésy qui produit des documents enre pouvant être analysés.

Nous avons pu ainsi déterminer dans chaque bande de fréquence la moyenne des valeurs maximales et minimales de la perte en dbs pour chaque sujet, à chaque profondeur Sans recourir à des analyses statistiques de type multivarié nécessitant des moyens de calcul considérables, nous avons pu confirmer la réalité des constatations expérimentales, notamment que la perte en conduction aérienne au niveau des fréquences graves n est pas une fonction *directe de la pression et que l audition* est proche de sa valeur normale autour de la fréquence 1 500 Hz.

Interprétation des résultats

Cette hypoacousie de transmission souligne l'existence d'une perturbation dans les mécanismes qui assurent le passage du son depuis le conduit auditif externe jusqu à l oreille interne. Ces perturbations liées au mélange gazeux et à l'hyperpression peuvent siéger

— au niveau des propriétés de l'ensembl tympano-ossiculaire en modifiant l'impédan du système.

— au niveau des fenêtres labyrinthique dont le jeu peut être altéré.

Nous nous proposons lors d'une prochain expérience de faire des mesures d'impédanc afin de confirmer éventuellement la premièr hypothèse.

Validité des résultats

Il importait de vérifier que cette baisse audi tive n était pas due à des artéfacts techniqu qui pouvaient être au nombre de deux

— *a*) les 10 mètres du cablage reliant le ca son à l audiomètre une expérience très simp a montré que celà était sans effet.

— *b*) Il fallait surtout s assurer que l'écou teur gardait ses propriétés dans le mélange g zeux utilisé et en pression.

Nous avons donc procédé grâce au Labor toire d'Acoustique Appliquée (Monsieur Co sain) du C.N.R.S. de Marseille, à la vérific tion des courbes de réponse de l écouteur ut lisé, en chambre insonore à l'air libre d'u part *en mélange O_2–He jusqu à 60 bars*, av une oreille artificielle type BK 4152.

Les résultats obtenus dans des conditio expérimentales rigoureuses ont montré qu pour tout le champ fréquentiel au-dessus d 180 Hz, une augmentation du niveau de pression acoustique émis par l'écouteur devait entrainer une correction en mieux de l'ordre de 10 à 18 dbs, suivant la pression (Fig. 3).

Ce niveau réel théorique de la perte audit en fonction de la pression a pu donc être déterminé en ajoutant cette valeur à la valeur moyenne expérimentale obtenue en audiométrique automatique dans chaque bande de fréquence testée (Fig. 4 5)

EXAMEN ELECTRO-NYSTAGMOGRAPHIQUE

Il n a été réalisé que pour Saturation I. Il e en effet connu que les fortes pressions ser

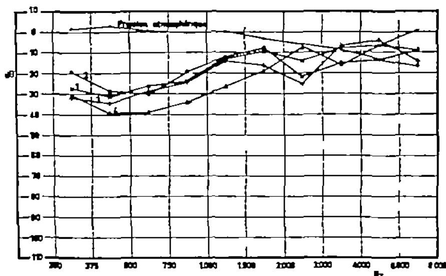


Fig. 4. Niveau réel théorique à 25 bars. Sujet Gauret. *1* 10 bars, *2*, 15 bars; *3* 20 bars; *4* 25 bars.

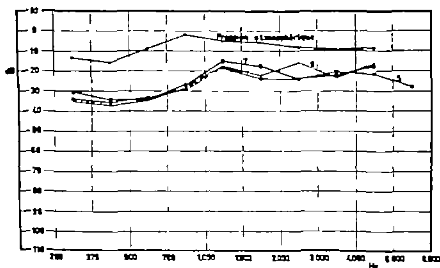


Fig. 5. Niveau réel théorique au-dessus de 25 bars. Sujet Le Pechon. *5* 30 bars; *6*, 35 bars; *7* 40 bars.

blent altérer la fonction vestibulaire, et il n a pas été jugé prudent de les tester à plus de 250 mètres.

Le protocole expérimental est voisin de celui de l'audiométrie appareil en dehors du caisson, test avant l'expérience, entraînement des plongeurs à faire une épreuve calorique à son camarade et vice versa.

Cette *stimulation calorique, dérivée de la* technique de Hallpike, est l'injection d'eau à 30 contrôlée par thermomètre Braun Tastomed H (précision inférieure à 0 15 C de 18 à 35) pendant 40 secondes, et à débit constant.

L enregistreur est un Dyno Graph R (coupleur 9 806 A) Beckman constante de temps 0,3 seconde.

L'E.N G est pratiqué après l examen audiométrique. On commence par l étude du regard

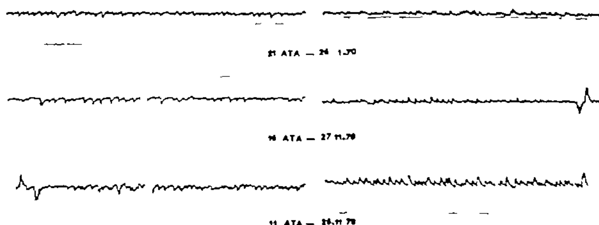


Fig 6 Exploration vestibulaire calorique, saturation I Technique de Hallpike. Température de l'eau, 30°C, irrigation continue durant 40 sec. Sujet Delemotte.

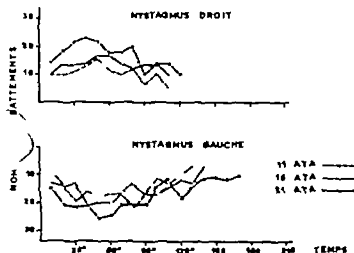


Fig 7 Exploration vestibulaire calorique, saturation I. Technique de Hallpike. Température de l'eau, 30°C irrigation continue durant 40 sec. Sujet Delemotte.

PARAMETRES	PRESSIONS					
	21 ATA		16 ATA		1[illegible] ATA	
	D	G	D	G	D	[illegible]
DUREE (secondes)	120	170	110	140	18	[illegible]
NOMBRE DE BATTEMENTS	205	301	144	210	7	[illegible]
FREQUENCE (bat/s)	1 7	1 7	[illegible]	1 3	[illegible]	[illegible]
AMPLITUDE	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
RYTHME	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
SENSATIONS VERTIGINEUSES	+++	+++	++	++		

en position normale dans différentes positions, yeux ouverts, et fermés dans la lumière ou dans l obscurité.

Puis le sujet pratique la stimulation. Il est laissé 6 minutes entre l'irrigation de chaque oreille.

RESULTATS

Sur les deux enregistrements, un seul permet une interprétation aux différents paliers testés.

On peut souligner

— l'absence de signes vestibulaires spont nés, notamment l'absence de nystagmus spo tané ou révélé.

— une réponse nystagmique à l'épreu thermique présentant certains caractèr quantitatifs particuliers, en effet les exame pratiqués à diverses profondeurs, objectivé une augmentation de la fréquence et de la d

rée du nystagmus provoqué, variant proportionnellement avec la pression (Fig. 6).

Cette perturbation objective est à rapporter avec la sensation vertigineuse subjective accompagnant l'examen, qui varie dans le même sens. Mais il n'est pas possible d'attribuer à ces perturbations une origine périphérique ou centrale.

L'interprétation de ce résultat unique est donc délicate on peut cependant souligner la possibilité d'une hyperexcitabilité vestibulaire relative et bilatérale en fonction de la profondeur atteinte susceptible de modifier le système nystagmogène.

Toutefois l'inégalité de la réponse nystagmique entre les deux oreilles existait chez ce sujet avant l'expérience (Fig 7)

CONCLUSIONS

Ainsi la plongée très profonde entraine des perturbations de l'audition et du système vestibulaire chez le sujet normal.

La perturbation vestibulaire au moment ou l'homme s'apprête à travailler sous la mer de manière de plus en plus hardie peut notamment avoir des conséquences graves. Un résultat unique est certes peu probant, mais nous avons eu peur de poursuivre cette expérience lors de Saturation II car à 400 m s'il y a le moindre incident, l'homme ne peut plus être secouru. Et si le Professeur Chouteau, lors d'expériences animales précédentes, a pu ramener indemnes deux boucs de 1 100 m fictifs à la surface, il y a eu chez d'autres animaux, et à de moindres profondeurs, des pertes d'équilibre sévères.

Ainsi des risques existent, qui ne peuvent être ignorés si des humains, même sélectionnés, quittent notre milieu naturel, que ce soit pour l'espace ou pour le fond des mers.

SUMMARY

Vestibular and auditory function are examined in four divers who went down to depths of 750-1 200 feet. The thresholds for airconduction went up those for bone conduction did not change. The vestibular function did not show any changes either

ZUSAMMENFASSUNG

Die Gleichgewichts- und Gehorfunktion beim Tauchen wurde untersucht bei vier Tauchern die bis zu einer Tiefe von 250-400 m hinabgestiegen sind. Knochenleitung und vestibuläre Funktion blieben unverändert. Die Luftleitung zeigte erhöhte Schwellen.

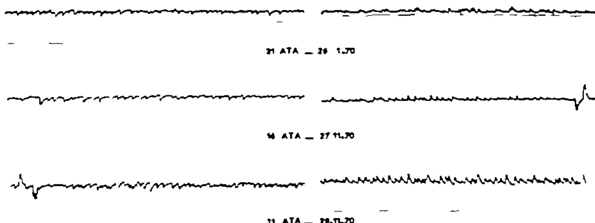
DISCUSSION

J Tonndorf My question concerns the calibration of Mr Appaix equipment, i.e. especially of his ear phones. I would assume that his audiometric findings could be explained as being caused by the change in the compliance of the air in the middle ear (to wit— no change for bone conduction!). This change in compliance should also affect the earphone and that prompted my question.

H Spoendlin. I would like to ask Mr Appaix whether he has also observed cases who, during slow decompression, experienced troubles resembling a Menière attack with vertigo, having loss and tinnitus. We have observed two such cases during an ordinarily slow decompression.

R. Hinchcliffe: Mr Tonndorf has already asked my question concerning the possibility that the observed changes in hearing were artefacts due to the effects of the pressure changes on the response of the ear phone. Incidentally what was the correlation coefficient for the comparison of pressure measures and hearing level?

A Appaix ((Réponses) L'audiomètre n'a pas été testé spécialement puisqu'il était hors du caisson. L'écouteur par contre à été testé très sérieusement et la modification de pression entraînait une facilitation du passage des sons. Il n'a pas été observé de vertige à la décompression chez l'homme. Il ne nous a pas été possible de pratiquer un E.N.G en raison du risque pour le sujet examiné. Chez l'animal nous avons constaté des troubles de l'équilibre à 400 mètres. A 250 mètres nous avons constaté un abaissement des seuils des fréquences basses. A 400 mètres ceux des hautes fréquences le sont également. Il n'y a pas de proportionnalité entre l'importance de l'abaissement des seuils et celle de la profondeur



G 1

Fig 6 Exploration vestibulaire calorique, saturation I Technique de Hallpike. Température de l'eau, 30°C Irrigation continue durant 40 sec. Sujet Delemotte.

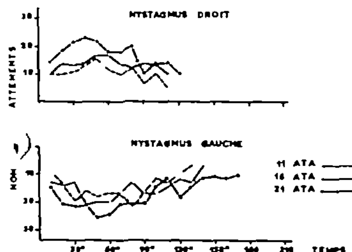


Fig 7 Exploration vestibulaire calorique saturation I. Technique de Hallpike Température de l'eau, 30°C, irrigation continue durant 40 sec. Sujet Delemotte.

[illegible]	PRESSIONS					
	21 ATA		16 ATA		[illegible]	
	D	G	D	G	[illegible]	[illegible]
[illegible] ([illegible])	120	170	110	140	[illegible]	[illegible]
[illegible] DE [illegible]	[illegible]	307	144	[illegible]	117	[illegible]
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en position normale dans différentes positions, yeux ouverts, et fermés dans la lumière ou dans l'obscurité.

Puis le sujet pratique la stimulation. Il est laissé 6 minutes entre l'irrigation de chaque oreille.

RÉSULTATS

Sur les deux enregistrements, un seul permet une interprétation aux différents paliers testés.

On peut souligner

— l'absence de signes vestibulaires spontanés, notamment l'absence de nystagmus spontané ou révélé.

— une réponse nystagmique à l'épreuve thermique présentant certains caractères quantitatifs particuliers en effet, les examens pratiqués à diverses profondeurs, objectivent une augmentation de la fréquence, et de la du-

ganes infectés, elle ne débute qu après l'apparition sensible de l'interféron.

L élévation de température a un effet favorable sur sa production qui est accrue quand existe un état d'immunité.

Par contre le stress a un effet défavorable sur ce système Si lors d'une primo-infection virale, c est l'interféron qui freine la multiplication du virus et réduit sa dissémination, lors d'une réinfection ce sont les anti-corps spécifiques presents dès l'entrée du virus dans la circulation qui ont le rôle principal, mais le système interféron peut toutefois servir de 2e ligne de défense

Utilisation de l'interféron en thérapeutique humaine

Si son utilisation à des fins thérapeutiques est justifiée et possible, l application pratique en clinique humaine rencontre à l'heure actuelle des difficultés presque insurmontables. En effet, il faudrait disposer d'interféron produit si possible par des cellules humaines et en grande pureté. En outre il faudrait en obtenir une concentration suffisante au site même de la multiplication virale Or l'interféron injecté et distribué dans tout l organisme disparait rapidement. Des administrations répétées seraient donc nécessaires. Enfin le traitement devrait débuter avant l'infection ou tout à son début.

Devant l'impossibilité pratique actuelle d'administrer l'interféron, les auteurs ont songé à induire artificiellement sa production chez les malades. De nombreux inducteurs ont été proposés, mais aucun, à l'heure actuelle, ne s est révélé satisfaisant soit à cause de la faible quantité produite soit à cause de la toxicité de l'inducteur

Comme j écrivais cela, j'oubliais que j avais craint à certains moments qu on ne pût répliquer et faire de l'interféron. Or peu de temps après, j apprenais que des chercheurs avaient même pu l'utiliser à des fins thérapeutiques. En effet dans une étude d'Edmond Marlon j'ai pu lire qu actuellement fonctionne en Angleterre un Comité scientifique de l'Interféron présidé par le Docteur Isaacs lui-même et en collaboration avec l' « Institut d'ophtalmologie de l'Université de Londres » Ce comité a demandé aux Laboratoires Wellcome de produire et tenir en réserve une certaine quantité d interféron en vue d'un traitement des cas de kératite vaccinale.

Cette expérience menée en 1960 par les Docteurs Cantell et Comila conduisit à l'efficacité d'un tel traitement.

On administra cette substance prélevée sur des cellules de singes rhésus et pour s assurer de l'inocuité du produit, on s adressa à un volontaire dont l'oeil devant être enlevé par suite d'un mélanome malin, mais dont la cornée et la conjonctive étaient normales On instilla pendant 2 jours et toutes les demi-heures une goutte dans l oeil du volontaire. Après quoi on procéda à l énucléation et on constata que les cellules épithéliales de la conjonctive n avaient rien d anormal. Ainsi rassurés, les praticiens se mirent au travail.

Ils traitèrent alors six cas de kératite vaccinale par une goutte toutes les demi-heures et pendant deux jours. Le lendemain tout était entièrement guéri alors que normalement on en arrivait à la perte de l'organe.

Il n'y en eut que six cas et c est trop peu pour affirmer qu'il s agit là d'un remède miracle, mais tous les espoirs sont permis, d'autant plus qu'il est probable que d'autres cas furent traités. Car si la vaccination et la sérothérapie sont efficaces, il y a tant de virus qu'il n est pas possible d'en faire la vaccination, souvent inopérante d'ailleurs.

Le Poids moléculaire de l'interféron serait de 63 000 alors que celui de l'hémoglobine est de 63 800 Rappelons que le poids atomique de l'hydrogène est consiéré comme égal à l'unité L'interféron est une macromolécule légèrement plus petite que celle de l'anticorps correspondant. Il est stable à 20 pendant de nombreuses semaines mais inactivé par le chauffage à 60 Il n'aurait aucune toxicité et ne provoquerait pas d'accoutumance. Son rôle n est pas de chasser les virus mais de les rendre inactifs, car les cellules traitées par lui accueillent normalement le virus, mais il cré

alors dans la cellule des conditions telles que le virus, quel qu'il soit, ne peut plus proliférer La difficulté est de pouvoir mettre le produit in situ, à l entrée de l antigène ou de l allergène

En ce qui nous concerne, il n y a pas de problème à ce sujet et nous avons le droit d'espérer que l'interféron sera efficace dans *tous les cas de rhume, de grippe, d'asthme même et ce, en instillant dans les narines ou* l'oro-pharynx quelques gouttes comme on l'a fait dans l oeil pour les cas de kératite vac cinale et pourquoi pas aussi dans certains cas d'anaphylaxie ou d allergie

Et puisque je cite ces deux termes je tiens à répéter ce que j'ai dit il y a quelques années au congrès du Collegium à Amster dam où je me suis insurgé contre l'habitude d'employer le mot d'allergie (von Pirquet, 1910) plutôt que celui d'anaphylaxie (Richet, 1902) En effet ce dernier est bien plus précis, puisqu'il signifie « le contraire de la protection ou de l'immunité » alors que le premier veut dire simplement « reaction ou action autre ou différente » De même souvent dans ces cas on parle d' « hypersensibilité » au lieu de « sensibilisation »

Enfin, après avoir écrit tuot cela, j apprenais que le 2 mai 1966, l' « American Society for microbiology » a fait l'étude de la stimulation de l'Interféron par l'agent anti-viral antibiotique le « Statolon » qui est un polysaccharide Cet effet est aussi produit par certaines bactéries, par des endotoxines bactériennes et par rickettsia.

Quant au Statolon il a été injecté dans le *péritoine de la souris et l'analyse de la production d'Interféron a été faite Son niveau maxi*mum est produit après 12 heures, se maintient jusqu à 71 heures pour décroître et atteindre environ 150 unités. Après 4 jours tout est terminé

La quantite d'Interféron produite dépasse 3 000 unités pour lesquelles il suffit de 190 µg de Statolon injecté On a fait également cette expérience sur la poule et certains primates avec les même résultats, mais bien entendu avec des doses plus fortes allant jusqu'à 2 500 µg.

Pour conclure disons que nous espérons que très bientôt l'interféron sera mis à la disposition de tous les praticiens !

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Acta Otolaryng 73 151–159 1972

GLUTAMATE, β-HYDROXYBUTYRATE AND SUCCINATE DEHYDROGENASES IN POST MORTEM INNER EAR FLUIDS

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Abstract Polyacrylamide and agarose gel electrophoresis was used to study glutamate (GluDH), β-hydroxybutyrate (HBDH) and succinate dehydrogenase (SDH) activity in normal human serum (NHS), normal cerebrospinal fluid (CSF) and perilymph from two cases of Menière's disease and in corresponding post-mortem fluids, brain tissue and liver homogenates. In post-mortem serum and in liver homogenate, one strong band of GluDH was found at the site between the mobility of LDH_4 and LDH_5 and this band did not appear in CSF or inner ear fluids. The slower bands present in serum were absent from post-mortem CSF and cochlear fluids. Activity corresponding to the sites of LDH_{1-4} was found in all fluids and control stainings. This was attributed to non-specific reduction of NAD by the electrophoretic media.

In a series of studies dealing with various inorganic and organic components of serum, cerebrospinal fluid (CSF), perilymph and endolymph, we have earlier reported on lactate (LDH) (Palva & Raunio 1969) and malate dehydrogenases (MDH) (Palva et al., 1970 Palva & Forsén, 1970) In post mortem serum, the LDH_4 and LDH_5 fractions were significantly larger than the corresponding percentages in CSF or cochlear fluids, the latter two showing nearly identical isoenzyme patterns. As for MDH, using the reaction order from malate to oxalacetate, a similar relationship was obtained, the values for MDH in serum being significantly higher than those for perilymph and CSF while the isoenzyme pattern of cochlear fluids and CSF was similar Using a reaction order from oxalacetate to malate the results were different and showed much lower activity values in serum and CSF compared with cochlear fluids. This reaction order gave results similar to those reported earlier by Schmidler & Schmeder (1966), and by Silverstein & Schuknecht (1966) It is possible that several of the MDH fractions reflect inclusion of endogenous substrates and the reaction quantity is probably affected by LDH in the direction from oxalacetate to malate.

Concerning other dehydrogenases van der Helm (1962 *a*, 1962 *b*) reported that there were four zones of L-glutamate dehydrogenase (GluDH) in brain extracts of human origin, whereas no activity was found in CSF He also found five fractions of β-hydroxybutyrate dehydrogenase (HBDH) in human brain, one of the fractions being extremely weak. In normal CSF three zones of activity were demonstrated.

Succinate dehydrogenase (SDH) activity has earlier been demonstrated histochemically in the hair cells in the organ of Corti by several investigators (i.e. Vosteen, 1956 Nakai & Hilding, 1968) This enzyme has not been demonstrated by immunological methods.

In an attempt to find further correlation between serum, CSF brain tissue and cochlear fluids, we have proceeded to make analyses of GluDH, HBDH and SDH activity in the above mentioned fluids and tissue homogenates.

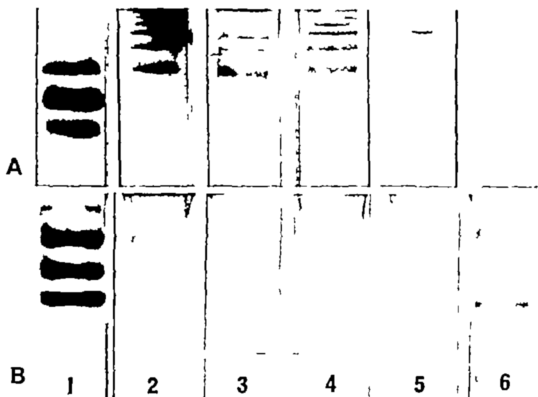


Fig 1 Polyacrylamide gel electrophoresis of NHS (undiluted) and normal CSF (concentrated 10 times). 20 μl of sample was used for each run. NHS (*A*) and CSF (*B*) electrophoresed in tris glycine buffer 1 – L, 2 – GluDH, 3 – HBDH, 4 – SDH 5 – staining in the presence of $MgCl_2$ and without substrate, 6 staining without $MgCl_2$ and without substrate. Bands at the sites of LDH_{1-5} are seen in all runs. The slow bands are haptoglobins.

MATERIAL AND METHODS

Perilymph was obtained after removal of the round window membrane and of the foot plate through the round window using a thin polyethylene tube and suction by mouth, from 55 temporal bones of fresh cadavers. When the vestibule appeared empty bone was removed from the promontory to expose the cochlear duct, and a sample of cochlear endolymph was collected by capillary force using small glass pipettes. Next, enough of the posterior rim of the oval window was removed to expose the utricle, which was then punctured with capillary pipettes to obtain samples of utricular endolymph A fourth specimen was obtained from the semicircular canals but this necessarily represented a mixture of perilymph and of utricular saccular as well as canal endolymph and cells. The amount of perilymph obtained was generally 20 μl while the amount of endolymph varied from 1 to 4 μl. CSF was obtained from the spinal canal and serum from the large thoracic veins. Brain homogenates were prepared using a method described earlier (Palva et al., 1971)

Polyacrylamide gel electrophoresis was performed in 7% gel by means of disc electrophoresis as modified by Dietz & Lubran (1967) The modification includes application of the samples in sucrose instead of in sample gel The amount applied was 20 μl for post mortem serum and brain homogenate diluted to 1 20 or sometimes 1 10, and 20 μl undiluted CSF and inner ear fluids. The inner

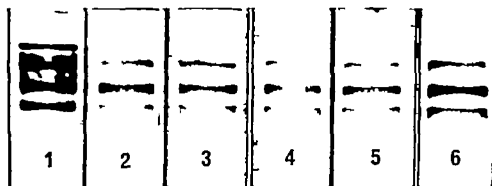


Fig. 2. Polyacrylamide gel electrophoresis of post mortem serum, diluted 1 10. Staining with $MgCl_2$ present, borate buffer amount of sample 20 μl. 1 – LDH 2 – GluDH, 3 – HBDH, 4 – SDH, 5 – $MgCl_2$, no substrate, 6 – no $MgCl_2$, no substrate. Bands developed at the site of LDH_{1-3} with all enzymes but they were much weaker than LDH isoenzyme bands. Staining without $MgCl_2$ resulted in stronger bands.

ear fluids had to be pooled in order to get sufficient quantities.

For detection of dehydrogenase activity the following staining solution was prepared NAD (10 mg/ml) 1 0 ml, NaCl (0 1 mol/l) 1.0 ml Phosphate buffer (0.5 mol/l, pH 7 4) 2.5 ml Nitroblue tetrazolium (1 mg/ml) 2.5 ml Phenazine methosulfate (1 mg/ml) 0.25 ml. Substrate solutions: Sodium lactate 1.0 mol/l, Sodium L glutamate 0.5 mol/l DL β-hydroxy butyrate 1 mol/l Disodium succinate 1 mol/l.

Each solution was adjusted to pH 7 4 and 1 ml of appropriate substrate solution was added to the staining solution. For detection of nonspecific staining the substrate solution was replaced by 1 ml of distilled water (control staining)

The buffer recommended for disc electrophoresis is tris glycine (Davis, 1964) Since it has been shown that glycine may act as a substrate for dehydrogenases (Ferguson, 1971), a tris borate buffer was also prepared in which borate ion (tris 5 mmol/l disodium tetraborate 0.02 mol/l) was substituted for glycine ion To discover whether magnesium had any effect on the nonspecific staining, some control stainings were carried out by adding 1 ml of $MgCl_2$ solution (5 mmol/l) to the staining solution with or without substrate.

Agarose gel electrophoresis was performed by Wieme's method modified by Palva & Raunio (1968, 1969) An amount of 3 μl of the sample was applied twice into the starting slit. In the case of perilymph the amount obtained from one temporal bone was generally sufficient for all runs. For endolymph, the samples from each temporal bone could be used for only one enzyme analysis because of the small volume of fluid available. Each fluid was used as such without concentration or dilution.

The isoenzyme bands were stained using the same staining solutions as described for polyacrylamide gel electrophoresis with the exception that KCN (0 1 mol/l) was substituted for NaCl.

Quantitation of isoenzyme staining was performed with Vitatron recorder at 536 nm.

RESULTS

Polyacrylamide gel electrophoresis

Analyses of GluDH, HBDH and SDH from NHS and normal CSF showed weak activity in the area corresponding to the activity of LDH_{1-3} bands. The control runs were also positive (Fig. 1). In the post-mortem serum (Fig. 2) brain homogenate, CSF and all inner ear fluids (Fig. 3), distinct bands appeared corresponding to the mobility of LDH_{1-3}. In

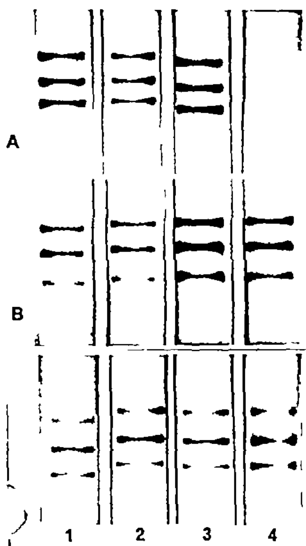


Fig 3 Isoenzyme analysis of post-mortem CSF (*A*) perilymph (*B*) and endolymph (*C*) on polyacrylamide gel using glycine buffer 1 = GluDH, 2 = HBDH, 3 = SDH, 4 = no substrate The bands corresponding to the mobility of LDH_{1-3} appeared in all test fluids and in the controls. In this CSF sample, the control showed weaker bands than obtained by staining in the presence of substrates. The amount of sample was 20 μl.

addition, there was varying activity in the slowly moving area, corresponding to the mobility of LDH_4 and LDH_5. The intensity of staining with all three substrates was generally similar in the case of all test fluids. In the control runs too, activity remained in the bands corresponding to the activity areas of LDH_{1-3}

Analyses of LDH in all the above test flui showed strong LDH_1–LDH_4 bands. Howeve these were very much stronger than the band observed with the three substrates used fo enzyme analysis in this study If the stainin of *NHS* isoenzymes was done without $MgCl_2$ the bands were much weaker In control run weak zones of activity remained at the area of LDH_2 and LDH_3 (Fig 4) After preincuba tion of post-mortem serum with purified LDH isoenzyme (Boehringer) strong bands o LDH_{1-4} *appeared with a predominance o* LDH_1 In control runs without substrate a trace of activity was seen in the bands cor responding to the mobility of LDH_{1-3} (Fig 4)

In neither NHS nor post mortem fluids, d addition of $MgCl_2$ to the staining solution intensify the bands in the control runs o the contrary some control stainings showed stronger bands without $MgCl_2$.

Substitution of tris borate for tris glycine buffer resulted in weaker LDH_{1-3} mobility bands in the control runs (Fig. 5).

In one patient with labyrinth destruction d to Menière s disease the perilymph runs sho ed all three activity bands when the substra for the three enzymes studied were used (F 6) Control runs could not be made becau there was not enough perilymph available.

Electrophoresis on agarose gel

Analyses of GluDH showed that in po mortem sera and in liver homogenates the was one very strong band which occurred b tween the sites of LDH_2 and LDH_3 and w much stronger than any of the other ban This band was not seen in any of the contr runs and thus must represent true GluD activity In CSF and in perilymph and end lymph analyses, activity appeared in the sit of LDH_{1-3} and this activity persisted in t control runs (Fig. 7)

Using substrates for HBDH and *SDH* dete tion the post-mortem serum analyses show five bands, one being slower than LDH_5. I CSF and inner ear fluids only the three fas

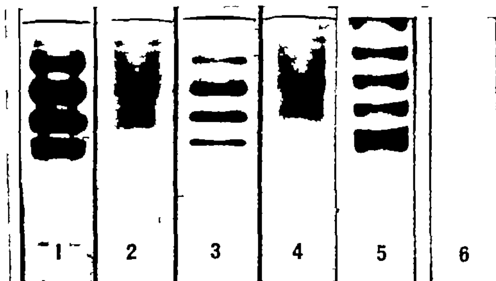


Fig. 4. LDH analysis of NHS (1–4) and post mortem serum (5–6) on polyacrylamide gel using glycine buffer. Sample size 20 μl. LDH staining 1 = with $MgCl_2$, 2 = control run without substrate, 3 = with substrate but without $MgCl_2$, 4 = without substrate and without $MgCl_2$, 5 = post-mortem serum preincubated with LDH_4, then analysed for isoenzymes, 6 = same without substrate. NHS controls showed the same pattern with distinct bands for LDH_1 and LDH_4. Omission of $MgCl_2$ made LDH bands much weaker. After preincubation with LDH_4 this band was prominent in post-mortem serum and the control showed traces of LDH_{1-3}.

moving bands appeared. However the control runs showed similar staining.

GluDH, HBDH and SDH analyses were also performed using NHS, normal CSF and perilymph obtained from another case of Menière's disease, the fluid being obtained during labyrinth destruction. Neither the test runs nor the control runs without substrate showed any enzyme activity (Fig. 9). Analysis of NHS and of normal CSF were negative in all analyses.

DISCUSSION

Only a few facts emerged from the innumerable tests performed in studying these three dehydrogenases. One was that post mortem serum and liver homogenate contain a distinct GluDH activity on a band situated between LDH_2 and LDH_3 in mobility. This strong band was never seen in control runs and undoubtedly represents GluDH activity. It is noteworthy that this band appears neither in post-mortem CSF nor in perilymph or endolymph. Another fact emerging from the results was that the slowly moving fractions, corresponding to the activity sites of LDH_4, LDH_5, and another slower band, appear only in post-mortem serum and not in CSF, perilymph and endolymph.

We were puzzled by the constant activity seen in the control tests with any of the described test procedures. This activity was apparently not due to endogenous lactate but a result of nonspecific reduction of NAD (Ferguson, 1971). The activity at the site of the slowest band in the post mortem serum dehydrogenase stainings is probably due to alcoholdehydrogenase (Dietz & Lubrano, 1971). In polyacrylamide gel, this activity apparently remains in the vicinity of the application site. The bands seen in the control stainings are not due to glycine alone (Ferguson, 1971) because they appear, though more weakly, when borate is substituted for glycine.

The discrepancy between our results and those obtained by van der Helm (1962 *a*, b) was

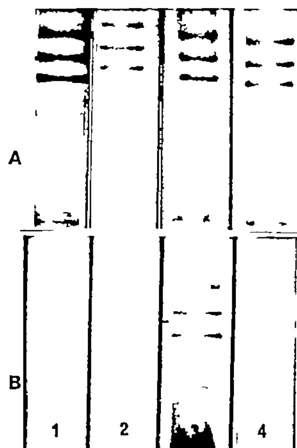


Fig 5 Isoenzyme analysis of brain tissue homogenate diluted 1 20 in glycine buffer (*A*) and 1 10 in borate buffer (*B*). The size of the sample was 20 μl. 1 = GluDH, 2 = HBDH, 3 = SDH 4 = no substrate Weaker dilutions in glycine buffer showed stronger bands than seen in borate buffer The same bands appeared with all substrates and in the controls without substrate

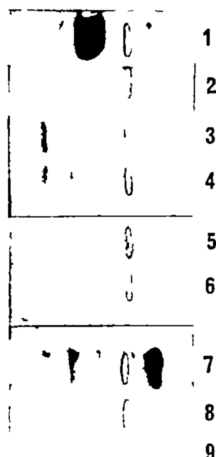


Fig 7 Agarose electrophoresis with GluDH staining of 1) post-mortem serum, 2) CSF 3) perilymph, 4) perilymph–endolymph mixture, 5) cochlear endolymph and 6) utricular endolymph Staining without substrate 7) post mortem serum, 8) perilymph, and 9) perilymph–endolymph mixture The amount of sample was 6 μl but for endolymph it was 3μl. In post-mortem serum there is a strong GluDH band corresponding to the mobility of LDH₁₋₃. LDH and LDH did not appear in inner ear fluids or CSF Anode on the left.

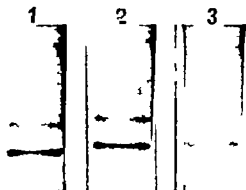


Fig 6 Isoenzyme analysis of perilymph obtained during surgery from a case of Ménière's disease. Glycine buffer amount of sample 10 μl. 1 = GluDH, 2 = HBDH, 3 = SDH. Bands corresponding to the mobility of LDH₁₋₃ are seen the one representing LDH was the weakest.

also puzzling He observed distinct GluDH and HBDH activity in the brain homogenate and in normal CSF The bands observed by him correspond well to the mobility of the bands shown by our two electrophoretic methods However while our control stainings were positive with the exception of one GluDH band, his controls were reported to be negative. As all other activity bands with the three dif

ferent substrates showed exactly the same mobility we are inclined to believe that there was no true GluDH, HBDH or SDH activity but the bands showed nonspecific reduction of NAD The strong single GluDH fraction in the post mortem serum was probably due to liver damage during the terminal stages of the disease and therefore appeared in serum but not in CSF or in inner ear fluids.

Our results are compatible with those of Falkenberg et al. (1969) who demonstrated that after tetrazolium salt staining of starch gel enzymograms of brain extracts containing NAD-dependent dehydrogenases, a pattern of LDH isoenzymes became visible although the incubation mixture contained no lactate but only the specific substrate for the dehydrogenase examined They also found that ex tracts of human tissue contained only a si

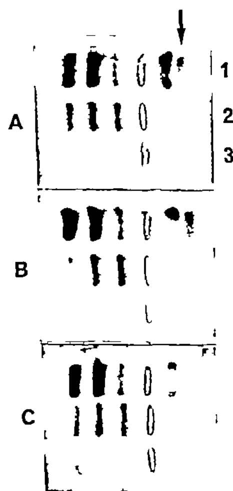


Fig. 8 Isoenzyme analysis of post-mortem serum, CSF and perilymph. (*A*) HBDH, (*B*) SDH, (*C*) control without substrate. Inner ear fluids and CSF did not show the slowly moving bands corresponding to the mobility of LDH₅, they also failed to show an even slower band (arrow) seen in post-mortem serum.

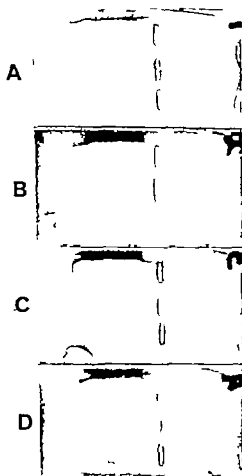


Fig. 9 NHS, normal CSF and perilymph from a case of Menière's disease analysed on agarose gel. Amount of sample 6 µL (*A*) GluDH, (*B*) HBDH, (*C*) SDH, (*D*) control. Using this sample size, no bands appeared in any of the normal test fluids or controls.

band of glutamate dehydrogenase with both starch gel electrophoresis and DEAE-cellulose chromatography In starch gel this band was also located between LDH_1 and LDH_2 bands According to Falkenberg et al (1969) the supporting media of the gel electrophoresis (starch polyacrylamide cellulose acetate, agar) *contain groups similar to lactate insolubly* bound to the polymerous gel system

No definite new information on the origin of labyrinthine fluids is obtained from the present study Specific bands due to these three enzymes do not seem to appear in detectable quantities in fresh fluids during life The terminal stages, which cause distinct alterations in the serum as far as GluDH is concerned, can be explained by liver damage and liberation of GluDH into the serum. This band and the nonspecific slowly moving fractions do not filtrate into CSF or inner ear fluids.

RÉSUMÉ

La distribution des quelques isoenzymes dans la périlymphe et dans l'endolymphe a été déterminée et une correlation a été faite avec la distribution des isoenzymes dans le liquide céphalo-rachidien et dans le sérum.

ZUSAMMENFASSUNG

Glutamat (GluDH), β-hydroxybutyrat (HBDH) und Succinatdehydrogenasen (SDH) Aktivitat wurden mit Elektrophoresis auf Polyakrylamid und Agarose aus normalem Serum, Zerebrospinalflüssigkeit, Perilymphe und besonders aus post mortalen Flüssigkeiten und Gehirn- und Leberhomogenaten untersucht. In post mortem Serum und Leberhomogenat wurde eine GluDH Fraktion gefunden, mit Mobilität zwischen LDH und LDH_2, die nicht in Zerebrospinalflüssigkeit oder Perilymphe und Endolymphe zu finden war Die kathodischen Fraktionen, regelmässig in post mortem Serum, erschienen nicht in anderen Flüssigkeiten. Aktivität auf die Regionen entsprechend LDH$_1$ wurde in allen Testflüssigkeiten und deren Kontrollen demonstriert. Diese Färbung war vermutschtlich durch eine nonspezifische Reduktion von NAD verursacht.

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Finland

DISCUSSION

U Fisch. You indicated that the cerebro-spinal fluid influences the perilymph in two ways: 1) the cochlear aqueducts, and 2) the perineural spaces. In view of the changes in protein content observed in the *perilymph of patients with acoustic neuromas* and the fact that the protein content of the CSF in the internal auditory canal may be very high in different conditions, as e.g. in progressive inner ear deafness, I wonder which way of communication between CSF and perilymph (the perineural or the aqueduct) is the most effective in the experience of Mr Palva

T Palva (Reply) to Mr *Fisch*. I agree that very often the protein concentration around the internal auditory meatus may rise even if the spinal tap reveals normal protein value. In cases of perception deafness, lacking definite data, I would say that both pathways, the cochlear aqueduct and perineural pathways are effective. In acoustic tumours, on the other hand, our recent work suggests that it is the cochlear aqueduct which functions as a pathway perineural channels being more or less blocked.

Acta Otolaryng 73 160–174 1972

RECENT REFINEMENTS OF QUANTITATIVE MICROCHEMICAL ANALYSIS OF TISSUES AND CELLS OF THE INNER EAR

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Abstract The application of the quantitative histo- and cytochemical method of Lowry to microscopic structures of the inner ear is illustrated in several examples. The total energy reserve and the metabolic rate of inner ear structures are estimated on the basis of measurements of metabolite levels at resting conditions and following ischemia. Recent technical refinements, such as the separation of the organ of Corti into cell layers and the dissection of single hair cells, are described and preliminary chemical results presented. Value and limitations of the approach for the study of pathological states are briefly discussed.

The minute size and complex structure of the auditory and vestibular end-organs pose a variety of methodological obstacles to the elucidation of their chemical properties. Fortunately one of the most powerful methods for the chemical analysis of discrete microscopic elements of brain and retina, the method of quantitative histo- and cytochemistry of Lowry (1953 1963) can be adapted to the specific requirements of the inner ear (Matschinsky & Thalmann 1967 *a* Thalmann et al., 1970 *a*). The approach consists of an integrated se-

This work was supported by US Public Health Research Grant NB 06575

Abbreviations.

ADP adenosine 5 -diphosphate
ATP adenosine 5 -triphosphate
GDH glutamic dehydrogenase
TPN triphosphopyridine nucleotide, oxidized
TPNH triphosphopyridine nucleotide reduced

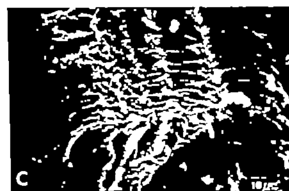
AW ampullar wall
CA, crista ampullaris
MS, macula sacculi
MU macula utriculi
OC, organ of Corti
RM Reissner's membrane
SCC semicircular canal
SV stria vascularis

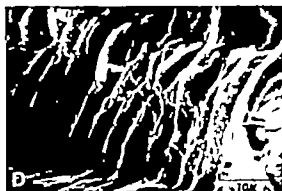
DC, Deiters cell layer
HC, Hensen cell layer
IHC inner hair cell layer
OHC, outer hair cell layer

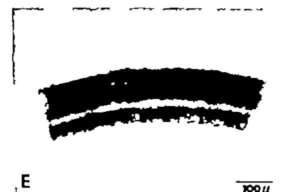
Fig 1 A Scanning electron micrograph of osmium fixed, freeze-dried organ of Corti *in situ* This and all following specimens are from the guinea pig, unless otherwise indicated.
(*B*) Similar preparation, dissected away from the modiolus and the basilar membrane (Fig. 1 *A* and *B* from Marovitz et al., 1970.)
(*C*) Scanning electronmicrograph of unfixed, freeze-dried organ of Corti There is some degree of artefact, but major structural features are clearly visible
(*D*) Scanning view into the tunnel of Corti (unfixed freeze-dried material). Outer tunnel rods, broken during dissection (see also Fig. 1 *F*), are in the upper right portion of the picture.
(*E*) Unfixed, freeze-dried organ of Corti viewed by transmitted light in the dissecting microscope. Major topographical landmarks are readily visible and the outlines of individual inner and outer hair cells may be distinguished (From Thalmann et al., 1970 *a*)
(*F*) Unfixed freeze-dried organ of Corti embedded in celloidin, sectioned at 5 *μ*, and stained with PAS hematoxylin. Overall structural preservation appears to be satisfactory
(*G*) Epithelial layer of macula sacculi dissected from an unfixed, freeze-dried temporal bone embedded in Epon, sectioned at 1 *μ* and stained. Both types of hair cells may be distinguished.
(*H*) Epithelium of crista ampullaris treated similarly to the specimen shown in Fig. 1 *G*


A
100 μ

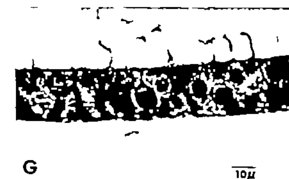

B
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C
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D


E
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F
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G
10 μ


10 μ

quence of preparatory and analytical steps designed to produce *in vitro* quantitative information which closely approximates *in vivo* conditions in the tissues In essence the rationale is as follows: Chemical processes are arrested with minimal delay by rapid freezing and remain suspended by subsequent freeze drying at low temperature. During processing the organ of Corti and other inner ear tissues retain their three-dimensional structure (Fig 1) and once dry may be exposed for reasonable periods of time to room atmosphere (at low relative humidity) for dissection The dry weight, determined on quartz fiber microbalances, is used as the index of sample size The high degree of sensitivity required for the analysis of enzymes and metabolites in the minute samples of inner ear structures (Fig 2) is achieved by means of inherently sensitive fluorometric techniques in combination with enzymatic cycling (Lowry et al., 1961) (Fig. 3) As evident from Fig. 2, there are other analytical methods available which cover at least part of the required range, but the method developed by Lowry offers the highest sensitivity in combination with wide applicability One of the most important features of the entire approach is the potential for measuring the tissue levels of various metabolic substrates and intermediates. Although enzyme profiles (in terms of maximal *in vitro* activities) provide information about the metabolic capacity of a given system they do not by themselves give indications about the extent to which the available machinery is actually being used More realistic estimates about the actual reaction rates occurring *in vivo* may be inferred from the steady state levels of the respective substrates and products. Furthermore changes of metabolite levels as a consequence of short term experimental interference may provide valuable information about dynamic metabolic patterns.

In the following presentation an attempt is made to illustrate with a few examples the potential of this approach for the study of the energy metabolism of the inner ear Recent

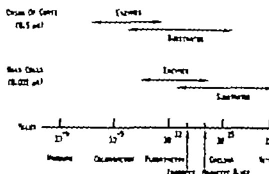


Fig 2 Estimated range of sensitivity needed available for the measurement of most enzymes *substrates involved in energy metabolism in diff* size samples of inner ear structures. Since enz are measured in terms of the amount of substrate verted over prolonged periods of time the analyt sensitivity required is much less than that requ for the determination of steady state tissue le of the respective substrates and products. (Mod after Lowry 1963 and Giacobini, 1970.)

technical refinements, which make possible study of different cell types of the organ Corti, are outlined. Possible future appli tions and limitations are discussed.

The first set of results was selected to monstrate the distribution of energy reser among inner ear structures and to illustr one possible approach to the estimation of *in vivo* metabolic rate of organ of Corti stria vascularis from the initial rate of dep tion of energy reserves under conditions ischemia

Table I shows the resting levels of the h energy phosphates ATP and P-creatine, those of the basic fuels, glucose and glycogen. Carbohydrate stores are high in all inner ear tissues, compared with brain. Particularly conspicous is the rich glycogen endowment of the organ of Corti The comparatively high glucose values for the organ of Corti might in part be due to accretion of glucose from the labyrinthine fluids. The stores of immediately available energy in the form of ATP and P creatine are rather similar in brain and in various inner ear structures.

In tissues depending upon carbohydrates as the only major foodstuff the total energy re serve in terms of equivalents of high energy

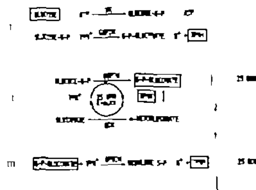


Fig. 3 Principle of enzymatic cycling after Lowry et. al., 1961, exemplified in the analysis of glucose. By means of auxiliary enzymes and cofactors, glucose is converted to glucose-6-P which in turn is converted to 6-P-gluconate, producing equimolar amounts of TPNH. TPNH exhibits native fluorescence; the amounts obtained in submicrogram samples however are too low for direct fluorimetry; therefore the TPNH produced in reaction (I) is introduced into the cycling system (II), where each pyridinenucleotide molecule is alternately oxidized and reduced in rapid succession. 6-P-gluconate and glutamate accumulate accordingly. The amount of 6-P-gluconate is finally determined in the indicator reaction (III). The TPNH formed in reaction (III) is a multiple of the TPNH formed in reaction (I) and is now measurable by direct fluorimetry. There are no theoretical limits in respect to the absolute amount that can be amplified, however the substance must be present at a concentration of at least 10^{-9}M to 10^{-4}M. Accordingly the initial reaction volumes must be kept very small. For volumes of less than 1 µl the assay is carried out under oil ("oil well" technique (Lowry 1963; Matschinsky et al., 1968)).

phosphates (both preformed and potentially available from anaerobic conversion of glucose and glycogen to lactate) may be calculated according to the following formula (Lowry et al., 1964) Sum ~ P = 2 ATP + P-creatine + 2 glucose + 2.9 glycogen (glycogen represented in terms of glucose equivalents; not accounted for in this simplified formula are minor contributions to the total energy reserve by ADP and various phosphorylated sugars). As seen in Table II the total energy reserve thus calculated is highest in the organ of Corti, considerably lower in stria vascularis and vestibular sensory epithelia, and by far the lowest in brain. The question now arises to what extent the distribution of energy reserves reflects the demands of the respective tissues. This problem has been approached in brain with the closed system method proposed by Lowry et al (1964). By interrupting the blood flow the brain is transformed into a closed system without significant exchange of metabolites and oxygen. Under these conditions the necessary energy can only be supplied anaerobically from the metabolites constituting the energy reserve. From the rate of depletion of these metabolites the use rate of high energy phosphate (i.e. the metabolic rate), can be calculated. Assuming that the energy consumption of the cells does not differ significantly from normal during the initial phase of ischemia, the initial use rate of high energy phosphate will constitute a reasonable estimate of the *in vivo* metabolic rate. Unfortunately, the situation is more complicated in the case of the inner ear since the closed system includes, in addition to the cellular structures, the large pool of labyrinthine fluids. Although dissolved oxygen is rapidly depleted in ischemia, sizeable amounts

Table I *Initial levels of principal metabolites constituting energy reserve in inner ear structures and brain*

	Organ of Corti	Stria vascularis	Macula sacculi	Macula utriculi	Crista ampullaris	Brain
Glucose	29.0	10.5	20.0	24.4	21.4	7.7
Glycogen	135	64	52.2	51.9	67.8	11.0
ATP	13.7	10.9	14.4	12.6	10.1	12.0
P-creatine	6.1	9.2	7.2	5.6	5.8	12.0

Values expressed in mmoles per kg dry tissue (brain values converted from wet weight using a factor of five). Cochlear data correspond to zero-time values from Figs. 4 and 5; vestibular from Th... 1971; data for brain from ... 1964 for adult, unanesthetized mouse.

quence of preparatory and analytical steps designed to produce, *in vitro* quantitative information which closely approximates *in vivo* conditions in the tissues. In essence the rationale is as follows: Chemical processes are arrested with minimal delay by rapid freezing and remain suspended by subsequent freeze drying at low temperature. During processing the organ of Corti and other inner ear tissues retain their three-dimensional structure (Fig. 1) and, once dry, may be exposed for reasonable periods of time to room atmosphere (at low relative humidity) for dissection. The dry weight, determined on quartz fiber microbalances, is used as the index of sample size. The high degree of sensitivity required for the analysis of enzymes and metabolites in the minute samples of inner ear structures (Fig. 2) is achieved by means of inherently sensitive fluorometric techniques in combination with enzymatic cycling (Lowry et al., 1961) (Fig. 3). As evident from Fig. 2, there are other analytical methods available which cover at least part of the required range, but the method developed by Lowry offers the highest sensitivity in combination with wide applicability. One of the most important features of entire approach is the potential for mea . the tissue levels of various metabolic substrates and intermediates. Although enzyme profiles (in terms of maximal *in vitro* activities) provide information about the metabolic capacity of a given system, they do not by themselves give indications about the extent to which the available machinery is actually being used. More realistic estimates about the actual reaction rates occurring *in vivo* may be inferred from the steady state levels of the respective substrates and products. Furthermore, changes of metabolite levels as a consequence of short term experimental interference may provide valuable information about dynamic metabolic patterns.

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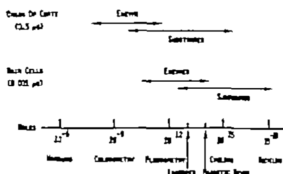


Fig 2 Estimated range of sensitivity needed and available for the measurement of most enzymes and substrates involved in energy metabolism in different size samples of inner ear structures. Since enzymes are measured in terms of the amount of substrate converted over prolonged periods of time, the analytical sensitivity required is much less than that required for the determination of steady state tissue levels of the respective substrates and products. (Modified after Lowry 1963 and Giacobini, 1970.)

The first set of results was selected to demonstrate the distribution of energy reserves among inner ear structures and to illustrate one possible approach to the estimation of the *in vivo* metabolic rate of organ of Corti and stria vascularis from the initial rate of depletion of energy reserves under conditions of ischemia.

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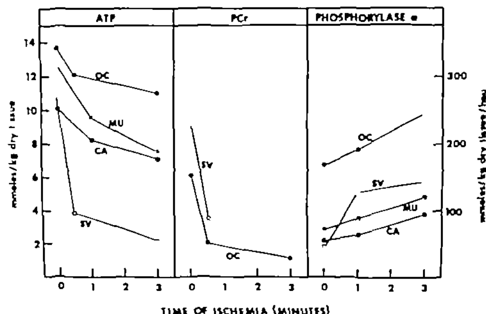


Fig. 4. Influence of short-term ischemia upon levels of ATP and P-creatine and upon activity of phosphorylase *a* in inner ear structures of the guinea pig. (Adapted from Matschinsky & Thalmann, 1967 *b*, 1970; Thalmann, 1971 *a*.)

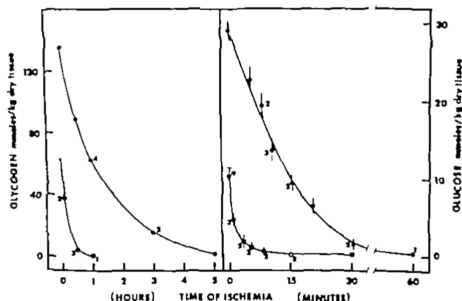


Fig. 5. Influence of ischemia upon levels of glycogen and glucose in stria vascularis (O) and organ of Corti (●) of the guinea pig. The means of the results for the indicated number of ears are recorded. In the case of glycogen, samples were obtained by sequential dissection from the entire cochlea, in some instances samples were analysed individually, in other cases all samples from a single ear were pooled together prior to analysis. (Data from Matschinsky et al., unpublished.) In the case of glucose, 14-16 samples of each structure, obtained from different regions of the cochlea, were analysed per ear. The vertical bars represent two standard errors of the mean. (Data from Thalmann et al., 1971 *b*.)

	OC	SV	RM	MS	MU	CA	AW	SCC

ENZYME ACTIVITY moles/kg dry tissue/hour

Fig 6. In vitro activity of enzymes involved in energy metabolism in different structures of the inner ear (Data compiled from Thalmann et al., 1970; Thalmann, 1970; Matschinsky & Thalmann, 1969 and Thalmann et al., unpublished.) (Abbreviations of enzymes: ALD = aldolase; CS = citrate synthase; GAPDH glyceraldehyde-P-dehydrogenase; Gly Syn = glycogen synthase; GOPDH = glycerol-1 P dehydrogenase; G6PDH = glucose-6-P dehydrogenase; HK = hexokinase; LDH = lactate dehydrogenase; MDH = malate dehydrogenase; PFK = phosphofructokinase; 6PGDH 6-P-gluconate dehydrogenase; 3PGK = 3-P-glycerate kinase; PGM = phosphoglucomutase; PHRL = phosphorylase; PHRLa = phosphorylase a; PK = pyruvate kinase; UDPG-PPHRL = UDPG-pyrophosphorylase.)

a major source of error since the decline rate of glucose in ischemia is much faster in the organ of Corti than in the fluids. This type of error could be evaluated by introducing a substance which will not pass the cell membranes (e.g. sucrose) into the labyrinthine fluids and measure the amount that can be recovered in the tissues.

Other examples of potential errors which would tend to *overestimate* the metabolic rate include the possibility that not all of the disappearing carbohydrate is converted into lactate and the possibility of a delay in the initial breakdown of glycogen in the organ of Corti, as suggested by the slow rate of activation of phosphorylase (Fig 4) (because of the exceptionally high glycogen levels in the organ of Corti, in combination with considerable biological variability and a slow rate of degradation, the initial rate had to be inferred indirectly by extrapolation from long-term experiments).

Finally it should be pointed out that the initial decline rates of ATP and P-creatine in anoxia may be even more rapid in stria vascularis than indicated in Fig 4 since in these experiments the initial delay in freezing amounted to several seconds and the shortest time of ischemia was comparatively long (30 sec). It is known that the main bioelectric responses of the cochlea start to decline within 2–3 sec of hypoxia, and consequently we are currently studying the effects of short-term hypoxia (and recovery thereof) by an experimental approach which guarantees rapid and

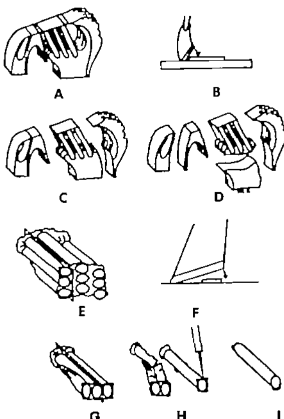


Fig 7 Schematic representation of the method for the separation of the organ of Corti into cell layers (*A–D*) and for the isolation of single hair cells (*E–I*).
(*A*) A radial section several cells thick is separated from a sample of whole organ of Corti.
(*B–D*) Successive steps in the separation of the five major cell layers. For better control of knife movement the tip is inserted into the Teflon dissecting block, and used as a fulcrum.
(*E, F*) A row of cells is separated from the outer hair cell layer.
(*G*) The selected hair cell is separated, the major portion of the sample must usually be sacrificed to produce one complete hair cell.
(*H*) The isolated hair cell is cleaned of debris with a fine needle.
(*I*) The final product is a reasonably clean and complete outer hair cell.

precisely controlled freezing rates and in which freezing may be accomplished with recording electrodes *in situ* at any given instant of an electrophysiologically monitored experiment.

Nevertheless, since the mentioned errors tend to operate in opposite directions, the presented preliminary calculations of the metabolic rate my be closer to the true value than anticipated. The metabolic rates calculated here are in fair agreement with estimates of the capacity of the citric acid cycle inferred from the maximal *in vitro* activities of citrate synthase, a regulator enzyme of the cycle (Fig 6) (Thalmann et. al., 1970 *b*) however the respective respiratory rates (Chou, 1964) and the capacities of Na K ATPase (Fig 6) (Kuijpers, 1969 Matschinsky & Thalmann, 1970) would suggest considerably larger differences in metabolic rates between stria and organ of Corti than observed here It should be remembered, though, that the maximal *in vitro* activity of enzymes does not necessarily reflect their actual *in vivo* performance

On this point we have discussed chemical processes as seen in whole tissues irrespective of longitudinal gradients and/or cellular diversity Average results provide guidelines, but may be misleading in the study of complex histological structures such as the organ of Corti. A strong longitudinal glycogen gradient has been demonstrated in the organ of Corti (Matschinsky & Thalmann 1967 *b*) (Fig. 7), and lesser gradients are apparent for glucose 6-P and P-creatine. How do such longitudinal chemical gradients of the organ of Corti correlate with the well known longitudinal anatomi-

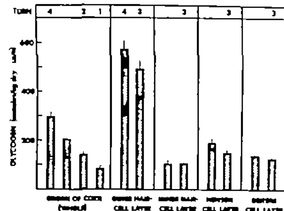


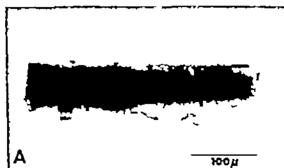
Fig 8 Levels of glycogen in different turns of the organ of Corti. Means and standard errors of the mean are recorded. (Adapted from Thalmann et al., 1970 *a*.)

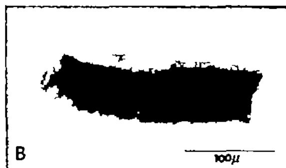
cal gradients (changes in relative size of different cell types) and with potential transverse chemical gradients (across different cell types)? This question and many others make it desirable to analyse different types of cells separately. A technique has recently been developed (Thalmann et al., 1970 *a*), which makes it possible to divide the freeze-dried organ of Corti into cell layers representing reasonably pure populations of the major cell types (Fig. 7). As yet only limited analytical data are available on cell layers separated in this way. As an illustrative example the distribution of glycogen within the organ of Corti is shown in Fig. 8 for the third and fourth cochlear turn. As anticipated on the basis of qualitative histochemical data (Falbe Hansen & Thomsen 1963, Ishii et al., 1969, Fig. 1 *F*) the outer hair cells exhibit a selective accumulation of glycogen reaching the extremely high concentration of 598 mmole/kg dry weight in the fourth turn and only sligthly less in the third turn. Since the outer hair cell layer is significantly contaminated by remnants of the Deiters cups and phalangeal processes (Fig. 9 *e*) which presumably are comparatively low in glycogen, it ... ely that the true glycogen levels in the ... cells are even higher. The data are not sufficient to throw light upon the question of the longitudinal glycogen gradient referred to previously.

In well preserved specimens the outer hair cell layer can be broken down further to yield short rows of hair cells or even single hair cells (Figs. 7, 10 *A–D*) (Thalmann et al., 1971 *a*). Similarly individual Hensen cells may be dissected, but so far it has not been possible to separate inner hair cells or Deiters cells. The dry weight of individual outer hair cells ranges between 0.3–1.5 ng, depending on the row and the turn from which the cell was obtained. The average amount of ATP in 3rd row outer hair cells from the 3rd cochlear turn was 1.13×10^{-14} moles of ATP per cell (S.E.M. $\pm 0.24 \times 10^{-14}$ moles for $n = 11$) corresponding to a concentration of 12.3 ± 2.6 mmoles/kg dry weight as opposed to an average concentration of 12.9 ± 1.4 ($n = 15$) for whole organ of Corti from the same region (Thalmann et al. 1971). It is evident that the variability is comparatively high for single cells but it is likely that this is due mainly to biologic variation or uncontrolled problems in the preparatory technique rather than to a lack of precision of the analytical technique since aliquots of ATP standards, carried through the same procedures, showed an error of less than 7%. The decline rate of ATP in ischemia was found to be similarly low in single hair cells and in whole organ of Corti and the question of why the cochlear microphonics decline so rapidly in ischemia therefore remains open.

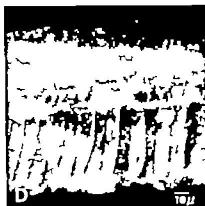
It is clear that as the level of resolution is increased, difficulties and the possibility of artefact increase at an even faster rate. It has already been indicated that the chemical analytical part of the entire approach is not usually the primary source of difficulty; more serious are problems involved in achieving a true representation of *in vivo* chemical conditions and adequate morphological preservation of specimens, an essential prerequisite to

Fig. 9 (*A*) Outer hair cell layer viewed by transmitted light in the dissecting microscope. Hair cell outlines are readily visible. A rudimentary fourth row can be seen just to the left of center.
(*B*) Similar specimen to that seen in 2 *A* but viewed from the side, showing the long axes of the hair cells.
(*C*) Scanning electronmicrograph of dissected outer hair cell layer prior to removal of the Deiters cups (Fig. 2 *A–C* from Thalmann et al., 1971 *a*.)
(*D*) Scanning electron micrograph of dissected outer hair cell layer with the major portion of Deiters cell remnants removed. (From Thalmann, 1971 *a*.)
(*E*) Unfixed, freeze-dried outer hair cell layer embedded in Epon, sectioned at 1 μ and stained. Remnants of the poorly-preserved Deiters cells and phalangeal processes are visible.
(*F*) Osmium-fixed surface preparation of organ of Corti, viewed by phase contrast at the level of the reticular lamina.
(*G*) Same preparation viewed by Nomarski differential interference contrast (DIC).
(*H*) Phase contrast view of the reticular lamina of unfixed, freeze-dried outer hair cell layer mounted in paraffin oil.
(*I*) Same preparation viewed by DIC.


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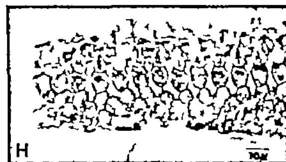

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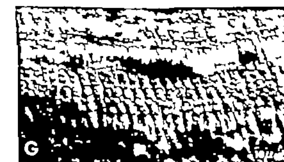

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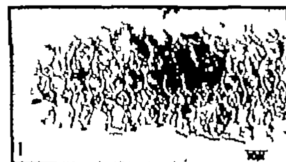

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H


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I

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In well preserved specimens the outer hair cell layer can be broken down further to yield short rows of hair cells or even single hair cells (Figs. 7 10 *A–D*) (Thalmann et al. 1971 *a*) Similarly individual Hensen cells may be dissected but so far it has not been possi[ble] to separate inner hair cells or Deiters cel[ls] The dry weight of individual outer hair cel[ls] ranges between 0.3–1 5 ng, depending on th[e] row and the turn from which the cell was ob[-] tained. The average amount of ATP in 3r[d] row outer hair cells from the 3rd cochle[ar] turn was 1.13×10^{-14} moles of ATP per ce[ll] (S.E.M $\pm 0.24 \times 10^{-14}$ moles for $n = 11$) co[r-] responding to a concentration of 12.3±2 mmoles/kg dry weight as opposed to an ave[r-] age concentration of 12.9±1 4 ($n = 15$) f[or] whole organ of Corti from the same regi[on] (Thalmann et al. 1971) It is evident that th[e] variability is comparatively high for sing[le] cells but it is likely that this is due mainly [to] biologic variation or uncontrolled problems [in] the preparatory technique rather than to a la[ck] of precision of the analytical technique, sin[ce] aliquots of ATP standards, carried through th[e] same procedures, showed an error of less tha[n] 7% The decline rate of ATP in ischemia w[as] found to be similarly low in single hair cel[ls] and in whole organ of Corti and the questi[on] of why the cochlear microphonics decline [so] rapidly in ischemia therefore remains open.

It is clear that as the level of resolution [is] increased, difficulties and the possibility [of] artefact increase at an even faster rate It ha[s] already been indicated that the chemical an[a-] lytical part of the entire approach is not usu[-] ally the primary source of difficulty mor[e] serious are problems involved in achieving [a] true representation of *in vivo* chemical condi[-] tions and adequate morphological preserva[-] tion of specimens, an essential prerequisite t[o]

Fig 9 (*A*) Outer hair cell layer viewed by transmitted light in the dissecting microscope. Hair cell outlines are readily visible. A rudimentary fourth row can be seen just to the left of center

(*B*) Similar specimen to that seen in 2 *A* but viewed from the side, showing the long axes of the hair cells.

(*C*) Scanning electronmicrograph of dissected outer hair cell layer prior to removal of the Deiters cups (Fig. 2 *A–C* from Thalmann et al., 1971 *a*)

(*D*) Scanning electron micrograph of dissected outer hair cell layer with the major portion of Deiters cell remnants removed. (From Thalmann, 1971 *a*.)

(*E*) Unfixed, freeze-dried outer hair cell layer embedded in Epon, sectioned at 1 µ and stained. Remnants of the poorly-preserved Deiters cells and phalangeal processes are visible.

(*F*) Osmium fixed surface preparation of organ of Corti, viewed by phase contrast at the level of the reticular lamina.

(*G*) Same preparation viewed by Nomarski differential interference contrast (DIC).

(*H*) Phase contrast view of the reticular lamina of [un]fixed, freeze-dried outer hair cell layer mounted [in] paraffin oil.

(*J*) Same preparation viewed by DIC.

the increase of resolution to the level of layer and single cell chemistry

Various sources of chemical and morphological artefact are discussed in detail elsewhere (Thalmann, 1971 *b*) a series of illustrations has been selected (Figs. 1 9 10) to exemplify the main approaches used in evaluating the morphological preservation of specimens and the quality of dissection New histological techniques, such as scanning electron microscopy and Nomarski interference contrast microscopy are valuable supplements to the more conventional methods. For the sake of comparison, some analogous results from fixed tissues are also shown. While it is comparatively simple to evaluate freezing and freeze-drying artefacts in normal ears, one may encounter serious problems when attempting to differentiate preparative effects from pathological changes, such as those induced by noise trauma or ototoxic agents. Damage due to freezing and freeze-drying may mimic certain pathologic alterations almost exactly (Fig 10 *G–L*) The incidence of true pathological change could be inferred to some extent by evaluating the opposite ear (which has been subjected to identical experimental conditions) with reliable histological techniques (e.g. the combined surface and cross-sectional technique of Bohne (1971)) It would probably be safer however to limit oneself to the study of chemical changes occurring under experimental conditions which are not severe enough to induce significant morphological changes (Eldredge et al., 1971)

Single hair cells can be isolated in the living state by enzymatic digestion of the organ of Corti with Pronase[R] (Goldstein & Mizukoshi, 1967) (Fig. 10 *E–F*) It should be possible to analyse these cells by means of techniques similar to those used by Hydén (1959) and others on native ganglion cells of the Deiters nucleus. It is clear however that such investigations would be limited primarily to the study of enzymes and other comparatively stable substances, and would not allow the evaluation of labile, low molecular weight compounds necessary to understanding dynamic metabolic patterns.

RÉSUMÉ

L'analyse biochimique des tissues de l'oreille interne a été projetée au niveau cellulaire. On a réussi à disséquer des couches cellulaires de l'organe de Corti et même des cellules ciliaires, tout en atteignant une conservation maximum des conditions chimiques in vivo. Par des méthodes ultramicrochimiques, des analyses quantitatives ont été effectués sur des enzymes et des métabolites. Des variations dans le taux des métabolites, observées pendant des interférences métaboliques, ont été comparées avec des résultats électrophysiologiques.

Fig 10 (*A*) Two unfixed, freeze-dried hair cells dissected free from the outer hair cell layer mounted in paraffin oil, and viewed by phase contrast.
(*B*) Same preparation viewed by DIC.
(*C*) Single freeze-dried hair cell isolated as indicated schematically in Fig. 7 mounted in paraffin oil, viewed by phase contrast.
(*D*) Same cell viewed by DIC. The preparation has been squashed slightly during handling.
(*E*) Single living outer hair cell isolated by proteolytic enzyme digestion (method after Goldstein & Mizukoshi, 1967), viewed by phase contrast.
(*F*) Same cell viewed by DIC.
(*G*) Unfixed freeze-dried organ of Corti from a normal guinea pig, embedded in Araldite, sectioned at 1 μ and stained. Note the swollen appearance of the second and third outer hair cells.
(*H*) Osmium fixed surface preparation of chinchilla organ of Corti (lower 2nd turn) from an animal subjected to noise trauma (108 dB octave band centered at 4 kHz, 3 hr) and sacrificed within 1 hour after exposure. The whole cochlea was fixed in osmium by perfusion and embedded in Araldite. Surface specimens were dissected from the hardened block (Bohne, 1971). In this preparation the reticular lamina appears fairly normal.
(*I*) At a lower level of focus, however the hair cells are seen to be grossly distorted.
(*J*) A thin section through the same area shows the swollen shape of the second outer hair cell. (Note the similarity to the third outer hair cell in Fig. 10 *G* which has been distorted during freezing and freeze-drying). (Fig. 10 *H–J* courtesy Dr Barbara A. Bohne.)
(*K*) Kanamycin-induced hair cell loss in guinea pig organ of Corti. This osmium-fixed, conventional surface specimen is viewed (by phase contrast) at a level approximately the same as that in Fig. 10 *I*
(*L*) Same preparation viewed by DIC. Note the distorted appearance of the two remaining hair cells of the first row A macrophage is seen in the upper left of the field (Fig. 10 *K L* from Kuhn et al., 1971).

ZUSAMMENFASSUNG

Die Anwendung der Methode der quantitativen Histo- und Zytochemie von Lowry auf mikroskopische Strukturen des Innenohres wird an Hand einiger Beispiele illustriert. Die Gesamt-Energiereserve und die Stoffwechselrate von Strukturen des Innenohres wird auf Grund von Messungen von Metabolitkonzentrationen unter Normalbedingungen und nach Ischaemie berechnet. Technische Verfeinerungen, wie die Aufteilung des Cortischen Organs in separate Zellschichten, sowie die Dissektion einzelner Haarzellen werden beschrieben und orientierende chemische Resultate gezeigt. Die Möglichkeiten und Limitationen der Anwendung der Methode auf pathologische Zustände werden kurz diskutiert.

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DISCUSSION

L Friedmann. In tissue cultures of the otocysts exposed to ototoxic antibiotics most enzyme systems remain fully active, except acetylcholinesterase. The cellular unit is perhaps too large and microsomal fractions might be more satisfactory Regeneration is of great importance when do the enzyme systems regain their activity? And is there any correlation between them and cellular morphology ultrastructure in particular

H Spoendlin. The primary decrease of ATP in the stria in anoxia, whilst the ATP content is maintained in the hair cells, is in line with our findings that the first structural changes occur in the stria and not in the hair cells. Very early however swelling of the dendrites to the inner hair cells is also observed and I would like to ask whether it would be possible to study separately the area below the inner hair cells, where these dendrites are. I think one could find early biochemical changes in this area. The difference in ATP-ase in the stria and the dark epithelium in the cristae might be due to the presence of blood vessels in the stria and their absence in the dark epithelium.

J Tonndorf Mr Thalmann mentioned that the newer chemical methods he is employing can assess rapidly occurring events. Since signal processing is involved in events taking place in msec or even *µsec*, I would like to ask him about the order of temporal magnitude of his chemical methods. It would be extremely important if one could correlate chemical and electrophysiological events on a common time base.

R Thalmann (Reply) to Mr *Friedmann* As I briefly mentioned before, enzyme levels are not generally sensitive indicators of dynamic metabolic patterns. In contrast to some of the labile metabolites, they usually remain essentially unchanged even in the presence of drastic metabolic interference. Lowry compares enzymes to the machinery present in a factory The available machinery provides an indication about the capacity of the factory for instance in terms of the maximum number of cars produced per day but by no means can we infer from the machinery alone how many cars are actually being produced normally or in emergency situations. Of course there are exceptions, for instance phosphorylase, an enzyme which exists in an active and inactive form, is rapidly inter convertible by various control mechanisms. Besides, there exist the phenomena of enzyme induction and repression, but these occur on a comparatively slow time scale

Concerning your second question about the potential of recovery or regeneration of the tissues. Apparently my remarks about the state of suspended animation induced by freezing and freeze-drying were misleading. These cells cannot be revived, due to severe damage to their fine structure the cells must be considered as an agglomeration of morphological artefact. However the chemical processes are, indeed, suspended and are closely representative of the conditions existing at the time of freezing. Chemical reactions may be rehabilitated *in vitro* but not in the organized and integrated temporal and spatial pattern characteristic of life.

We have not looked at the ultrastructural correlates of the observed chemical changes, but it is likely that as in other tissues swelling of the mitochondrial cristae would occur within ten minutes of anoxia or so. Studies on microsomal fractions would, indeed, be very informative but because of the small size of the inner ear structures there seems to be no easy way to obtain subcellular fractions from reasonably homogenous tissues by conventional methods. A possible way perhaps would be to use microsomal fractions of larger structures, not necessarily from the ear as carriers for small amounts of radioactively marked inner ear tissues. An alternative of high potential are studies of ribosomal activity *in situ* as initiated by Kraus at the University of Munster

To Mr *Spoendlin.* It would be interesting to study the synapses of the inner hair cells, but I do not envisage a reliable technique of identifying and separating them under the stereomicroscope. Since the inner hair cells are surrounded closely by supporting cells, we have not even succeeded in obtaining clean samples of populations of inner ear hair cells. So far the inner hair cell layer has been least representative of a specific cell type. Incidentally I do not believe that the present technique could or should be extended to the subcellular level. Morphological preservation of intracellular structure is poor and even the whole nucleus may be displaced, without this being recognizable in the stereomicroscope.

Regarding the dark cells of the vestibular labyrinth: It is difficult but not impossible to separate the epithelial layer of the ampullar wall and of other areas containing dark cells from the connective tissue, but there is no way of isolating pure populations of dark cells, except perhaps by a cross-sectioning technique.

To Mr *Tonndorf* Unfortunately the answer is 'no There is no hope that the extremely rapid chemical events connected with excitation and transmission can be detected by this method. You are talking about events occurring within milliseconds and less. What I mean by 'rapid' in the context of detectable dynamic events of energy metabolism is, most optimistically in the order of 10 seconds or so for labile substances such as P-creatine, ATP and inorganic phosphate. By contrast, metabolic changes detectable by

conventional qualitative histochemical techniques are in the order of 30 minutes or longer. Although the described chemical methods are by themselves sufficiently precise to detect changes in metabolite levels of a few per cent, the actual measurable differences are higher since dynamic chemical patterns have to be reconstructed from results of numerous animals, with the ensuing problem of biological variability. The physiologist is in a far more favorable position, since he can frequently obtain a tremendous amount of information from one single preparation with the added advantage of an inbuilt control.

It should be stressed once more that in our work we are not concerned directly with the chemical counterpart of information transfer in the sense of a 'biochemical theory of hearing' as claimed by Vinnikov and Titova. All we are attempting to measure are auxiliary processes which are essential in providing the energy required for a proper functioning of the processes that are directly involved in the transformation of auditory information.

Acta Otolaryng 73 175–182, 1972

DÉPOUILLEMENT STATISTIQUE D'UNE ENQUÊTE SUR L AUDITION DU PREMIER ÂGE

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Abstract Les réactions auditives de trois lots de sujets, 183 prématurés, 404 nouveau-nés, 1 644 nourrissons (2 231 sujets, 3 317 examens) ont été étudiées. Le protocole d'examen comportait une liste de matériel sonore et une liste des réactions observées comme preuve de la réponse auditive. Les résultats ont été obtenus après traitement des informations par le Laboratoire d'Etudes Mathématiques du Centre d'Energie Atomique de Pierrelatte. Ainsi peuvent être définis les stimuli les plus réactogènes pour la fonction auditive et les réactions les plus fréquemment observées sous l'effet d'un stimulus auditif Des âges optima d'examen sont ainsi définis. Une analyse de l'attitude du clinicien devant les techniciens de l'informatique est présentée.

Nul ne conteste actuellement la nécessité du dépistage précoce de la surdité chez l enfant, condition première d'une réadaptation bien conduite. Il convient de déterminer un protocole le plus adapté à ce dépistage Trois sortes de problèmes se trouvent posés *a*) celui des réactions observées sous l effet de stimuli sonores, *b*) celui du matériel sonore *c*) celui de l'âge optimum du dépistage.

Lorsque le protocole a été minutieusement mis au point et correctement appliqué, il reste, pour en interpréter les résultats avec certitude, à faire la part des conditions dans lesquelles s est déroulé l'examen. Autrement dit, il faut déterminer l'importance de facteurs tels que l'état de vigilance de l'enfant, l'heure du repas précédent et savoir quelles sont les modalités les plus favorables pour obtenir des réponses nettes.

Le travail que nous présentons a été poursuivi pendant cinq ans. Le nombre d'enfants examinés permet de dépasser le stade de la simple observation clinique. Pour définir les normes du protocole de dépistage, il était indispensable de se soumettre à un contrôle statistique strict. Aussi, est-ce à l'ordinateur que nous avons donné la parole, en lui confiant le dépouillement du matériel d'étude accumulé.

PROTOCOLE

Le protocole comporte l'étude des lots d'enfants, les techniques d'exploration de la fonction auditive, et les modalités de l'exploitation statistique.

MATÉRIEL

Quatre lots d'enfants ont été étudiés. Les âges sont exprimés en jours, à partir de la date des dernières règles, et non à partir de la date de la naissance. La date de naissance normale se situe, avec cette notation, au 287e jour

Lot numéro 1 183 prématurés, soit un total de 628 examens auditifs, les âges extrêmes sont de 140 et de 371 jours. L âge moyen est de 251 jours.

Lot numéro 2 404 nouveau-nés, soit un total de 880 examens auditifs. L examen a été pratiqué habituellement deux fois entre le premier et le huitième jour après la naissance

Lot numéro 3 940 nourrissons, soit un total de 1 022 examens auditifs. Les âges extrêmes sont de 259 et de 1 260 jours.

Lot numéro 4 704 nourrissons, soit un total de 737 examens auditifs. Ces enfants sont observés dans le cadre des examens systématiques d'un centre de Protection Maternelle et Infantile Les âges extrêmes sont de 280 et 1 015 jours.

Ainsi l enquête porte sur 2 325 jeunes enfants, avec un total de 3 317 examens. Chaque lot d'enfants a été examiné par la même équipe comportant un médecin audiophonologiste, et deux assistantes orthophonistes.

Technique d'exploitation

Chaque équipe use d'un protocole général d examen, mais ce protocole présente des adaptations à la catégorie d enfants qui lui est confiée Le protocole comporte la liste du matériel sonore, et la liste des réactions de l enfant aux stimulations auditives

Chaque stimulus est produit toujours dans les mêmes conditions. Les réactions de l enfant sont observées par au moins deux des testeurs

Avant de procéder à cet examen, il faut tenir le plus grand compte des conditions dans lesquelles il va se dérouler Ces conditions sont soigneusement notées milieu dans lequel se ouve l enfant, position de cet enfant heure repas précédant l examen, état de vigilance l enfant. Il faut également prendre en considération et ceci importe surtout pour les prématurés, l'état général de l enfant au moment où l examen est réalisé Les circonstances pathologiques peuvent modifier complètement les réactions de l'enfant il faut les connaître pour interpréter correctement les résultats.

Le rythme des examens varie suivant les catégories pour les prématurés, chaque enfant est examiné en moyenne toutes les trois semaines, certains grands prématurés étant vus à des intervalles un peu plus rapprochés, de une ou deux semaines.

Les nouveau-nés sont examinés entre le premier et le huitième jour suivant leur naissance, si possible deux fois.

Les nourrissons sont examinés une fois, à un âge variant entre quinze jours et deux ans et demi L examen n est répété que lorsque la première exploration n a donné que des résultats douteux.

Exploitation statistique

L observation est consignée sur une fiche spéciale permettant un traitement informatique. Cette fiche comporte deux parties, une première réservée aux renseignements généraux cliniques, une seconde qui se rapporte uniquement à l'examen auditif

Exploitation des résultats

Cette exploitation a été réalisée par le Bureau d'Etudes Mathématiques du Commissariat à l'Energie atomique de Pierrelatte L ordinateur était un I.B M. 360-50 Le programme était le BMD programme d'analyse multi-dimensionnelle (multivariate analysis–biomedical computer program)

COMMENTAIRE STATISTIQUE

Un certain nombre de réflexions s'impose avant d entrer dans l étude analytique par catégorie

Tests et réponses

Pour un test positif il peut exister une, deux, trois, ou même au maximum, quatre réponses positives différentes. Il convient donc de connaître très exactement pour un stimulus déterminé, non seulement le nombre de tests positifs, et le nombre total de réponses positives, mais aussi le nombre de tests positifs, et le nombre total de réponses. Selon que l'on considère le nombre de réponses positives, ou le nombre de tests pris globalement, on parlera de première ou de deuxième hypothèse.

Homogénéité et répartition des réponses et des tests

Il est important d étudier l'homogénéité et la *répartition des réponses* et des tests, en fonction des différents facteurs pris en considération, ainsi le sexe, le numéro de l examen, le milieu, la position, le repas, l'état de vigilance. On parvient ainsi à regrouper des lots pour

lesquels les conditions sont rigoureusement identiques. Ces lots doivent être numériquement suffisants pour qu une étude statistique puisse être effectuée.

Corrélations entre les réponses

Lorsque la corrélation est positive, deux réponses ont un très grand pourcentage de chance de se manifester simultanément. Si au contraire, la corrélation est négative il y a très peu de chance pour deux réponses d'apparaître simultanément. Cette notion est importante pour juger des différents stimuli et pour parvenir à la notion d'un protocole épuré.

Segmentation

Nous entendons par là l'étude de la ressemblance des stimuli vis-à-vis des réponses qu'ils déterminent. Les stimuli vont se regrouper L étude de la segmentation peut être réalisée selon deux attitudes d esprit mathématique

Première attitude On tient compte de la force des réponses. Le regroupement est donc, non seulement affaire de similarité, mais aussi d'efficacité.

Deuxième attitude On ne tient pas compte de la force de la réponse. Les stimuli se regroupent selon la similarité des réponses obtenues.

Il faut ajouter encore qu'il est possible de faire intervenir les corrélations entre les réponses. Les résultats qui tiennent compte des corrélations sont vus avec métrisation. Les autres sont donnés sans métrisation.

Les résultats avec métrisation nous ont montré que le calcul statistique ne peut pas tout résoudre. Il est nécessaire d'adopter une attitude conforme à la réalité des phénomènes observés. Lorsque des résultats sont offerts, il convient de réaliser un véritable choix. Pour réaliser les segmentations, il ne convient pas de prendre les corrélations telles quelles, mais de les discuter à la lumière des notions de physiologie connue.

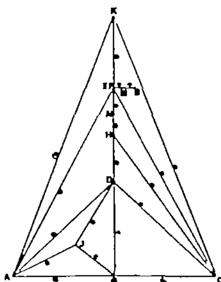


Fig 1 Catégorie 1 Corrélations.

ÉTUDE ANALYTIQUE PAR CATÉGORIE

Catégorie 1

Répartition des types de réponses par tranche d'âge et par stimulus. Cette étude précise l évolution des réponses en fonction de l âge Le *réflexe cochléo-palpébral* (A1–A2) *croît* très vite avec l'âge, le sursaut (C1–C2–C3–C4) moins vite. Les réponses du type éveil, agitation, clignement des yeux décroissent avec l'âge. En outre, les réponses se diversifient nettement à partir de la trentième semaine

Les réponses les plus importantes sont les réflexes (cochléo-palpébral ou musculaire) l'éveil et les réactions comportementales (agitation, grimaces)

On peut mettre en évidence le rendement des différents stimuli on note chez la prématuré le bon rendement des intensités fortes et de la voix.

Corrélations Elles montrent que les réponses A et C (réflexe cochléo-palpébral et sursaut) sont toujours correlées négativement avec les autres réponses; leur présence s oppose donc en moyenne à la présence d'un autre type de réponse quel que soit le stimulus employé. On pourrait émettre l'hypothèse d'un circuit

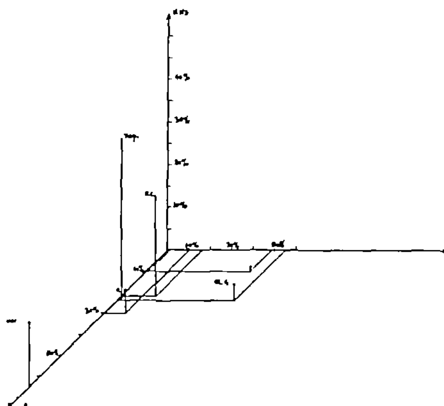


Fig 2 Catégorie 1 non normée. Protocole de dépistage.

neurologique prépondérant, inhibant la mise en jeu d autres circuits.

egmentations Le regroupement des stimuli ...on les réponses qu ils déterminent diffère selon qu'il est réalisé avec ou sans métrisation. Le regroupement le plus intéressant est celui obtenu sans métrisation. On isole ainsi six groupes de stimuli *intensités fortes, intensités* faibles, bruits familiers les fréquences aiguës (sifflets et clochettes) se rapprochent en deux groupes.

L'analyse discriminante montre que les réponses peuvent aussi être rapprochées et l'on forme ainsi trois groupes.

Le choix des six stimuli et la notation de ces trois groupes de réponses permet de définir un protocole simple pour le dépistage de la surdité chez le prématuré.

Analyse multidimensionnelle Elle précise l'influence des différents facteurs susceptibles de modifier les réponses obtenues sous l'effet des stimuli, en particulier état de vigilance, numéro d ordre de l'examen, âge de maturité, type de stimulus.

L étude de l'influence des stimuli met en évidence deux notions importantes sur le plan de la maturation auditive

Selon l'intensité du stimulus l enfant réagit très tôt aux intensités fortes, plus tardivement *aux intensités faibles* *Par exemple* le tambour est efficace à forte intensité chez les grands prématurés. A faible intensité, il ne le devient qu au voisinage du terme normal.

Selon la fréquence l'enfant réagit plus précocement aux sons graves qu'aux sons aigus. Ainsi avec les sifflets et la clochette la plus aiguë dont les sons fondamentaux se situent au delà de *2 000 Hz*, il faut *attendre* le voisinage du terme pour obtenir 90 % de réponses.

L influence de l âge de maturité est surtout manifeste pour certains stimuli globalement, c est à partir du 250e jour c est-à-dire du huitième mois, que l on obtient les meilleurs taux de réponses c est à cet âge ou au delà

que se situe donc le meilleur moment pour le dépistage.

L analyse souligne de plus l'importance du numéro d'ordre de l examen Il existe une croissance très nette du taux des réponses positives du premier au deuxième puis du deuxième au troisième examen, pour atteindre ensuite une sorte de phénomène de saturation. Il apparaît que le numéro d'examen explique mieux la variance que l'âge lui-même en précisant évidemment que ce numéro porte en lui une notion d'âge moyen auquel il est généralement pratiqué.

Cette influence du numéro d'examen met en évidence un phénomène remarquable dans le développement de la fonction auditive *le rôle de l'environnement sensoriel* du bain sonore dans lequel un grand prématuré se trouve plus précocement plongé qu'un enfant né à proximité du terme normal.

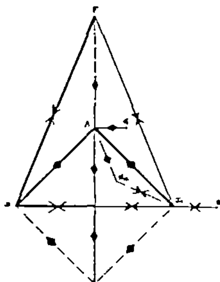


Fig 3 Catégorie 2. Graphe des corrélations entre les différents types de réponses.

— — — Probabilité entre 90 et 95 %. ——— Probabilité entre 95 et 99 %. ——— Probabilité supérieure à 99 %. × Corrélation positive. ♦ Corrélation negative.

Catégorie 2

Répartition des types de réponses par tranche d'âge Prenons l'exemple des réactions A1 et A2, c'est-à-dire réflexe cochléo-palpébral isolé, et réflexe cochléo-palpébral entretenu. A1 semble évoluer de façon régulière dans le temps, A2 au contraire présente manifestement une acmé au deuxième, troisième et quatrième jour Il est possible d interpréter de deux façons ce résultat. On peut admettre que l'enfant passe par une phase de sidération consécutive au traumatisme obstétrical, puis par une phase d adaptation avec une réactivité excellente puis la troisième est celle de l'habituation. Il est possible aussi de formuler une autre hypothèse et de parler de seuil d excitabilité en fonction d une maturation de l'appareil auditif La segmentation en âge légal ne correspond pas à la réalité des phénomènes, il convient de parler en âge de maturation.

Corrélations entre les réponses. Deux réponses de même catégorie ont un coefficient de corrélation moindre que deux réponses de catégories différentes, en d autres termes deux réponses passant par des circuits neurologiques différents ont plus de valeur que deux réponses qui empruntent le même circuit.

Analyse multi-dimensionnelle Cette analyse permet de connaître exactement le rôle des différents facteurs dans la corrélation des réponses sous l'effet des différents stimuli. Nous nous sommes particulièrement intéressés au sexe et au numéro d'ordre de l examen, au milieu, à l'état de vigilance de l'enfant. L analyse multi-dimensionelle a comporté deux études, l'une en tenant compte de l âge de maturité, l'autre en tenant compte de l'âge légal.

Ainsi retient-on quatre groupes de réactions. Un protocole pour la catégorie 2 pourrait comporter le tambour à forte intensité, le tambour à faible intensité, le sifflet et la voix. Il serait plus juste de dire qu'un protocole pour la catégorie 2, devrait comporter quatre types de stimuli. Un stimuli grave fort, un stimuli grave faible, un stimuli dans une zone de fréquence moyenne, enfin un bruit familier la voix étant certainement le meilleur

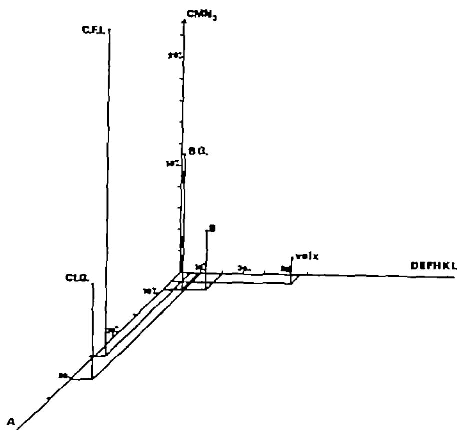


Fig 4 Catégorie 2, Protocole de dépistage.

Catégories 3 et 4

...istence de deux lots dans une même ... est intéressante, car elle permet d'étudier l'influence du testeur

Corrélations Le tableau des corrélations est intéressant, il montre particulièrement que le réflexe d'orientation présente une corrélation négative avec une probabilité supérieure à 99% avec le réflexe cochléo-palpébral, une corrélation négative aussi avec une probabilité comprise entre 95 et 99% avec le sursaut, mais il présente une probabilité comprise entre 95 et 99% avec les sourires.

Segmentations Si l'on ne tient pas compte de la force des réponses, les stimuli se regroupent selon la similarité des réponses obtenues, il est possible de faire intervenir les corrélations entre les réponses Les résultats qui tiennent compte des corrélations sont dits avec métrisation, les autres sont donnés sans métrisation. Les résultats montrent que les regroupements sont très intéressants sans métrisation. Avec métrisation, la segmentation prend une forme très particulière Le regroupement le plus intéressant est celui sans métrisation, qui isole les stimuli à forte intensité grave, les stimuli à faible intensité, et, enfin, les bruits familiers.

Analyse multi-dimensionnelle L'étude du pourcentage de réponses en fonction de l'âge de maturité est intéressant à considérer La voix obtient immédiatement 100% De très nombreux stimuli présentent des courbes parallèles à l'axe des abscisses. Seules les clochettes moyennes semblent montrer une évolution très nette Ce fait doit les faire éliminer d'une batterie de tests La même étude avec la deuxième hypothèse permet d'isoler le tambour à forte intensité, et la tasse-cuillère

L'analyse multi-dimensionnelle permet ainsi de trouver des stimuli à forte réactivité la

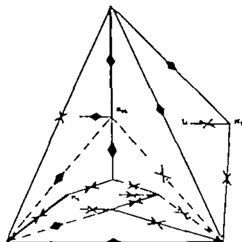


Fig. 5. Catégorie 3 + 4. Graphe des corrélations entre les différents types de réponses.

—·—·— Probabilité entre 90 et 95 %. ——— Probabilité entre 95 et 99 %. ——— Probabilité supérieure à 99 %. Corrélation positive. ◆ Corrélation négative.

voix, les clochettes aiguës, les clochettes graves, et les castagnettes.

On note un maximum de réponses respectivement vers 700 jours et 800 jours pour les deux hypothèses.

L'influence du testeur a été recherchée. Le testeur apparaît très significatif avec une influence de 15 %. Le sexe et le numéro d'examen ne sont pas significatifs.

L'âge est très significatif il semble présenter une influence maximum sur le taux de réponses à 700 jours où on enregistre le taux de réponses maximum un peu inférieur à 95 %. En moyenne, un seul enfant sur vingt ne répond pas.

CONCLUSIONS

La richesse du matériel constitué par cette étude, la multiplicité des variables en cause et leur interdépendance fréquente en rendaient l'analyse statistique indispensable. Encore fallait-il que les résultats dégagés fussent à leur tour discutés à la lumière des données physiologiques connues pour conduire cette recherche à son terme, s'est affirmée la nécessité d'une confrontation constante de ces deux ordres de réflexions, mathématiques et cliniques.

ANNEXE (GRAPHIQUES)

Stimuli

1. Tambour faible intensité (TfI)
2. Tambour forte intensité (TFI)
3. Bongo faible intensité (BfI)
4. Bongo forte intensité (BFI)
5. Cymbale faible intensité (CfI)
6. Cymbale forte intensité (CFI)
7. Clochette grave (ClG)
8. Clochette moyenne (ClM)
9. Clochette aiguë (ClA)
10. Sifflet grave (S.G.)
11. Sifflet aigu (S.A.)
12. Castagnettes (C)
13. Tapotements (Tap.)
14. Jouets de caoutchouc (J.C.)
15. Bruits de verre et de biberon (B)
16. Tasse-cuillère (T.C.)
17. Bruit de papier (B.P.)
18. Voix-chuchotement (Voix)
19. V.B. filtered white noise audiometer (V.B.)
20. Néometer Zenith (S)

Différentes réactions observées

A1 Réflexe cochléo-palpébral isolé
A2 Réflexe cochléo-palpébral en retenu
E1 Contraction généralisée sans sursaut
C1 Sursaut généralisé
C2 Sursaut localisé Moro
C3 Sursaut localisé tête
C4 Sursaut localisé membre
D1 Réaction d'éveil par ouverture des yeux quelques secondes
D2 Réaction d'éveil complet, agitation
D3 Réaction d'éveil complet, et pleurs
E1 Réaction d'arrêt
F1 Arrêt des pleurs
G1 Agitation généralisée
G2 Agitation localisée mouvements MS
G3 Agitation localisée mouvements MI
H1 Sourire
J1 Grimaces
J2 Arrêt de succion
K1 Clignement des yeux
L1 Mouvements de succion-ouverture de la bouche
M1 Déclenchement des pleurs
N1 Latéralisation vers la source des yeux
N2 Latéralisation vers la source de la tête
N3 Latéralisation vers la source orientation-investigation
P1 Refuge vers la mère
R1 Aucune réponse

SUMMARY

The report is based on the study of hearing in 2 231 cases, of which 183 were premature babies, 404 new-born babies and 1 644 from 15 days to 2 years

old (2 231 cases; 3 317 examinations). The sounds used were musical instruments, the human voice, the noises of the Auditory Screening Test and two L.F generators, one of which produced a single frequency and the other producing filtered white noise. The reactions of the children were analysed and classified. Elementary reflexes, Orientation, Waking reactions and Changes of behaviour An attempt at establishing a correlation between reaction and stimulus is made. A procedure for the systematic detection of deafness in children is proposed, giving the kind of sound stimulus to be administered and the reactions to be watched for The best age for this detection is also indicated.

ZUSAMMENFASSUNG

Ein protokolares Aufspüren der Schwerhörigkeit bei Kindern is realisiert worden bei Untersuchungen von 2 231 Kindern, wovon 183 Frügeburten waren, 404 Neugeborene 1 644 Säuglinge im ganzen 3 317 Untersuchungen der Gehörfunktion. Die Ergebnisse haben wir nach der Überarbeitung der Informationen durch das mathematische Labor des Energie-Zentrums von Pierrelatte erhalten. Diese Forschungen erlauben die Feststellung der sensoriellen Defizite.

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DISCUSSION

S Iurato Two questions: 1) How long does it take to perform this examination 2) What do you think about the opportunity to associate—at least in particular cases—more reliable tests, like E.R.A. or electrocochleogram.

G Lidén. I want to emphasize how important it is to detect deafness in the very young child. In order to initiate the right type of training/educational program even at an age of one year we also need to have a threshold audiogram of the child. At the Oslo-Collegium we presented such a method, i.e. visual reinforcement audiometry My question is how precisely *can you diagnose different degrees of deafness* with your method?

D Hood. I would like to take up with Professor Mounier-Kuhn the significance of the early detection of deafness in children. It is of course held that there exists a so-called critical period when hearing is vital to the acquisition of speech. Bench has reviewed the literature on the subject in a paper published in a recent issue of *Sound* the British Journal of Audiology and concludes that although certain periods may be of significance there is no evidence for the notion of a critical period. That may well be misleading and perhaps too much importance is attached to it. I should appreciate *Professor Mounier-Kuhn's* views.

P Mounier-Kuhn (Réponse) à Mr *Hinchcliffe* A Mr Hinchcliffe qui préfère à la segmentation un autre mode d'analyse statistique je dirai que la segmentation nous a *été* proposée par les ingénieurs de l'informatique. Je n ai pas d'opinion personnelle.

A Mr *Iurato* La durée de l'examen peut être très variable selon l'âge et les circonstances. Je pense que 30 à 45 minutes est un délai moyen raisonnable. Naturellement, l'École Lyonnaise qui est au premier plan pour l'étude de l'audiométrie electro-encéphalographique, l'utilise dans toutes les circonstances favorables ce qui n est pas toujours le cas.

A Mr *Lidén* Votre système paraît très intéressant et je l'étudierai à l'occasion.

A Mr *Hood* me demandant s'il y a une période critique pour l'acquisition du langage, je dirai qu'aux environs de 6–9 mois après la naissance l'enfant est particulièrement sensible à la voix humaine.

Acta Otolaryng 73 183–189 1972

CENTRAL COMPENSATION OF RETROLABYRINTHINE VESTIBULAR LESIONS

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Abstract The present study is based on the results of repeated vestibular examinations in a group of patients with retrolabyrinthine lesions. Central vestibular compensation is found to be based on the three following mechanisms: 1 *Accommodation* This mechanism seems to depend entirely on the integrity of the efferent vestibular system. 2. *Recovery* 3 *Central regulation.* Impaired peripheral vestibular function is substituted by optic-visual and somato-sensory mechanisms. It seems to depend mainly on the functional integrity of the cerebello-vestibular fibre connections.

In a previous paper (Pfaltz & Kamath, 1970) we put forward the concept that central compensation of a peripheral vestibular dysfunction is based upon two entirely different mechanisms:

The first one is a *specific vestibular phenomenon* depending on the integrity of the efferent vestibular system. It is termed "*accommodation*" i.e. by means of the efferent fibers the responses from the remaining intact labyrinth are modulated, adjusting them to the functional lesion (inhibition and facilitation). Accommodation is generally completed within a short time unless there is a lesion of the elements of the vestibular ganglion or an impairment of conductivity of the peripheral neuron. Functional disorders at the level of the vestibular ganglion or of the first neuron will implicate incomplete and delayed accommodation. *Recovery* of the vestibular endorgan is modifying this phenomenon.

The second mechanism is a *non-specific central phenomenon* substituting the impaired vestibular functions by optic and somatosensory regulation. Hence the term central compensation should strictly speaking only be used for the phenomenon of *central substitution.* It is dependent upon the functional integrity of the central nervous system. For this reason we may assume that traumatic and vascular lesions of the brain will delay the achievement of this compensatory mechanism according to the severity of the central disorder However we think that the term of *central compensation*—generally speaking—includes both the specific and the unspecific phenomenon: *Accommodation* with or without *recovery* and *central substitution* by optic and somatosensory regulations.

In the present study we have attempted to obtain more information about the influence of retrolabyrinthine lesions upon *vestibular accommodation.* We have also been particularly interested in the effect of a well-defined lesion of the central nervous system upon the specific and non-specific compensation mechanisms.

MATERIAL

Bell s palsy: 40 cases

Acute unilateral loss of vestibular function. 19 cases (vestibular paralysis of sudden onset)

Central lesions: unilateral cerebello-pontine angle tumour: case no 1

Unilateral neurinoma of the IX and X cranial nerves with ventral compression of the brainstem case no 2

Unilateral destruction of a cerebellar hemisphere: case no 3

Table I. *Stages of vestibular compensation*

Stage 1. Functional loss	
Spontaneous nystagmus Positional nystagmus Galvanic nystagmus, DP	Normal side
Stage 2. Accommodation	
Progressive suppression of spontaneous and positional nystagmus→normal side	
By inhibition, decrease of amplitude and frequency	
Progressive suppression and equalisation of responses of galvanic DP→normal side	
By decrease of the amplitude of nystagmus→normal side	
By increase of frequency of nystagmus→lesion (liminal and supraliminal responses)	
Stage 3. Central compensation	
Accommodation	
Recovery	
Reversal of	
spontaneous nystagmus→lesion	
galvanic resp. DP→lesion	
Substitution by optic and somatosensory regulation	

METHODS

We have used the same technique of vestibular examination and the same criteria for the evaluation of the results as published in our previous paper (Pfaltz & Kamath 1970).

RESULTS AND DISCUSSION

Bell's palsy

In Bell's palsy the occurrence of vestibular symptoms is well known. In a previous paper (Robert & Pfaltz, 1970) we reported that 75% of our patients, affected by an idiopathic facial paralysis, showed objective symptoms of a transient vestibular dysfunction.

In the majority of our cases we have found a great number of abnormal responses such as a raise of the galvanic threshold and a directional preponderance towards the unimpaired side during the first stage of the paralysis.

An increased threshold of the perstimulatory galvanic nystagmus must be interpreted as the symptom of a lesion of the first vestibular neuron, because it is never observed in endorgan lesions. For those reasons we have assumed that in Bell's palsy the vestibular symptoms may be interpreted as the expression of a mild and incomplete *concomitant lesion of the first vestibular neuron* within its common course with the facial nerve (i.e. eventually within its meatal segment).

Accommodation starts at a rather early stage, *recovery* of vestibular function is nearly always complete, and hence *central vestibular compensation* of this transient lesion of the

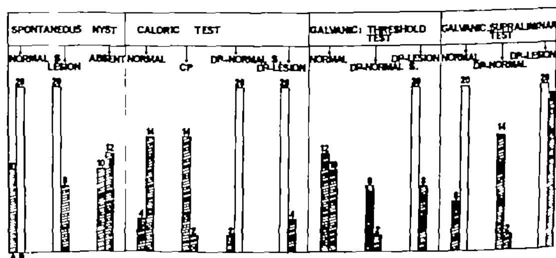


Fig. 1. Bell's palsy: survey of 40 cases. (*A*) Early stage (5–25 days interval between onset of paralysis and examination). (*B*) Advanced stage (26–240 days interval). CP: caloric hypoexcitability; DP: directional preponderance. ▤ positive; ☐, negative.

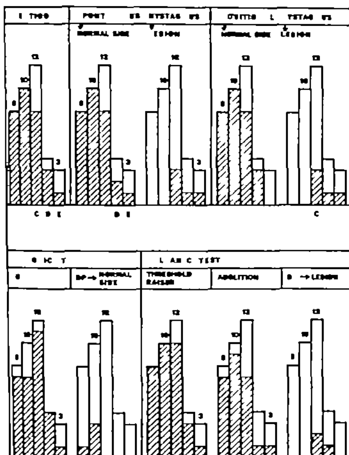


Fig. 2. Vestibular paralysis of sudden onset (unilateral lesion, normal hearing). Survey of 19 cases. Observation period: (*A*) < 1 week. (*B*) 1–4 weeks. (*C*) 1–6 months. (*D*) 6–12 months. (*E*) > 1 year ☐ positive ☐, negative.

peripheral vestibular neuron will be achieved within a very short time On the other hand an irreversible and complete lesion of the first vestibular neuron takes much longer to be compensated (Pfaltz & Kamath, 1970).

Vestibular paralysis of sudden onset (acute loss of unilateral vestibular function)

With respect to its pathogenesis, this type of vestibular dysfunction forms a rather inhomogeneous group. Dix & Hallpike (1952) reported on 100 cases whose chief symptom was vertigo, usually but not always paroxysmal in character and distinguishable from Menière's disease by the conspicuous absence of cochlear signs and symptoms. Their conclusion was the following:

"It seemed then attributable beyond any doubt, to some form of organic disease confined to the vestibular apparatus and localized, in all probability to its peripheral nervous pathways, up to and including the vestibular nuclei in the brainstem " The authors had chosen the term "*vestibular neuronitis*" for this particular vestibular dysfunction because they required a term comprehensive enough to encompass this uncertainty " i e the uncertainty of specifying the particular elements of the neurons, cells or fibres which were affected However there is little agreement concerning the precise delineation of what constitutes "vestibular neuronitis" (Coats, 1969)

Lindsay & Hemenway (1956) have published a case, the symptomatology of which was corresponding to that of vestibular neuronitis. In temporal bone sections they have found distinct degeneration changes in the vestibular nerve as well as in the vestibular ganglion. Accord

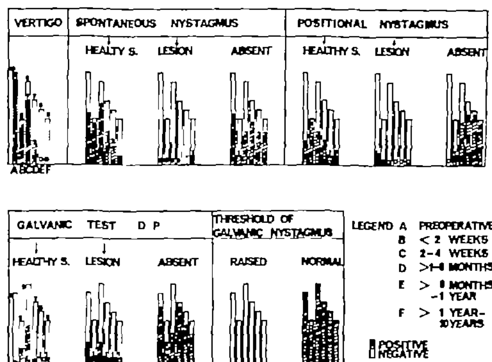


Fig 3 Labyrinthectomy (10 cases). Vestibular findings pre- and postoperatively

ing to their histological findings, the pathology ... to be attributed to a thrombosis of blood vessels within the internal auditory meatus. Later on, Lindsay (1967) preferred to reserve the term of "vestibular neuronitis" for patients with a vestibular disturbance " ... of inflammatory origin, in which definite localizing signs are lacking ... " Hart (1968) has chosen the term of "vestibular paralysis of sudden onset" which is more non-committal such as Frenzel's "acute loss of vestibular function" (1961)

With respect to its symptomatology our own material corresponds entirely to that of Dix & Hallpike (1952) Moreover in the majority of our cases we have observed abnormalities of the galvanic test, strictly indicating a lesion of the first vestibular neuron thus confirming Dix & Hallpike's hypothesis of a neuronal lesion. However in our material we could not find any evidence for a non specific or viral inflammatory process hypothetically responsible for this particular vestibular disturbance

Repetitive controls of those patients have shown that *accommodation* and *central substitution* of this particular group of vestibular dysfunction requires a considerably longer period than isolated lesions of the vestibular endorgan.

Central lesions

Three patients with distinctly localized lesions of the brainstem and the cerebellum have been examined several times before and after neurosurgical treatment. Our observations may be summarized as follows

In *combined peripheral and central vestibular lesions* (case 1—unilateral cerebello-pontine angle cholesteatoma—removed without section of the VIII nerve), peripheral symptoms will disappear first, because as soon as the cause of the lesion has been removed without damaging the first vestibular neuron,

accommodation of the peripheral functional loss will set in immediately as long as the vestibular lesion is confined to the endorgan

Central vestibular lesions

Substitution of central lesions by optic and somatosensory regulation mechanisms and control of activity of primary vestibular centres by inhibition and facilitation is a process which takes much longer than accommodation of an endorgan lesion. This has been observed in all our cases of *brainstem and cerebellar lesions* (cases 1 2, 3) In those patients, vertigo, disequilibrium and ataxia as well as the symptoms of a central vestibular disturbance (gaze nystagmus, positional nystagmus, disinhibition of experimentally induced nystagmus by caloric and galvanic stimulation) have been observed for a period of more than 13 months following the removal of the primary lesion, and they are most spectacular in a case of unilateral destruction of a cerebellar hemisphere

CONCLUSIONS

1 Transient and incomplete blockage of the peripheral vestibular neuron (e.g. Bell's palsy) does not result in severe disturbances of equilibrium because neither accommodation nor recovery are delayed.

2. Unilateral acute loss of vestibular function, a disturbance closely related to vestibular neuronitis, is apparently based upon a severe lesion of the peripheral vestibular neuron In some cases there is even some evidence that also the vestibular nuclei are involved. Accommodation as well as recovery take much longer to set in than in endorgan lesions. This may be explained by an irreversible lesion of the efferent vestibular system, annihilating the modification of the responses from the remaining intact labyrinth, which no longer can be adjusted to a unilateral functional vestibular deficiency

3 In combined peripheral and central vestibular lesions of common origin, peripheral symptoms disappear rather rapidly as long as the endorgan and not the peripheral neuron is impaired. This is due to a prompt onset and achievement of accommodation, whereas recovery may not always be complete.

4 Both subjective and objective symptoms of central vestibular disorders disappear far more slowly than those of endorgan lesions. Most of those central vestibular symptoms are caused by a disturbance of the voluntary gaze system, located within the brainstem (gaze nystagmus), or they are due to cerebellar lesions, e.g. the flocculo-nodular lobe (positional nystagmus, disinhibition of caloric and galvanic responses), or to lesions of the cerebellar cortex (ataxia, dyscoordination of movement) The cerebellar cortex provides " proper translation of the spatial concept of movement (existing in the cerebral cortex) into time (duration of innervation) This translation is necessary for rapid movements which are preprogrammed because they are too fast to be regulated continuously " (Kornhuber 1971). Brodal (1964) in his fundamental work on the anatomy of the central vestibular system has confirmed that cerebello-vestibular connections are far more abundant than vestibulo-cerebellar connections. Thus the vestibular nuclei are influenced from the vestibular part of the cerebellum, the flocculo-nodular lobe, as well as from the cerebellar regions, often referred to as spino-cerebellum (Vermis), since they receive the bulk of afferent impulses from the spinal cord.

In one of our cases (no 3) half of the cerebellar hemisphere had been destroyed by gunshot and had to be removed neurosurgically In that particular patient we have observed the most severe vestibular symptoms of central origin persisting longer than 13 months. If we keep in mind that one of the main properties of the cerebellum is supposed to be the transformation of spatial into temporal patterns (Braitenberg, 1961), we may understand why any partial or total interruption of the cerebello-vestibular connections will result in a severe disturbance of equilibrium.

Because centrally integrated spatial information is lacking, the vestibular nuclei will no longer be able to act as a coordination centre For those reasons also the substitution of vestibular functional impairment by optic and somatosensory regulations will fail as long as the primary vestibular centres are not under the control of higher order neurons.

RÉSUMÉ

Dans ce travail les auteurs présentent les résultats d'une étude sur la compensation vestibulaire des lésions retrolabyrinthiques. La *compensation centrale* des troubles vestibulaires se base sur les 3 mécanismes suivants. 1 *Accommodation.* Il s'agit d'une modulation de la réactivité de l'organe sensoriel intact par moyen des fibres efférents, soit par inhibition, soit par facilitation. 2. *Récupération.* 3 *Régulation centrale* Il s'agit d'une substitution des fonctions vestibulaires périphériques déficitaires par moyen de régulations optique-visuelles et somatosensorielles. Ce mécanisme régulateur semble être lié à l'intégrité des fibres cerebello-vestibulaires.

ZUSAMMENFASSUNG

In der vorliegenden Arbeit werden die Ergebnisse von Untersuchungen über das Verhalten der zentralen [vesti]bulären Kompensation bei retrolabyrinthären [Stö]rungen besprochen. Die *zentrale vestibuläre Kom[pen]sation im weiteren Sinne* umfasst folgende 3 Vorgänge: 1 *Akkommodation.* Das Zustandekommen derselben hängt weitgehend ab von der Integrität des efferenten vestibulären Systems. 2. *Erholung.* 3 *Zentrale Regulationsvorgänge* (zentrale Kompensation in engerem Sinne). Diese beinhalten im wesentlichen die Kompensation einer irreversiblen peripheren vestibulären Funktionseinbusse durch optisch-visuelle sowie *somato-sensorische Regulationsmechanismen. Ihr* Zustandekommen scheint auf Grund der vorliegenden Untersuchungsergebnisse von der Integrität der cerebello-vestibulären Verbindungen abzuhängen.

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DISCUSSION

U Fisch. Did you observe a bilateral suppressi[ve] effect following a sudden unilateral loss of vestibu[lar] function? We have observed this effect in the majori[ty] of patients having had vestibular neurectomy Th[e] bilateral effect may be of practical importance b[e]cause so-called "bilateral" neuronitis may be in fa[ct] unilateral losses with a bilateral inhibitory effect.

R. Hinchcliffe: Could I please make two observa[tions]: Ten years ago I studied a series of ov[er] 100 patients with vestibular disorder *All patien[ts]* were examined with pure tone audiometry caloric te[st] and galvanic test, as well as other tests. Some of th[e] patients who had normal audiograms and impaire[d] caloric responses showed normal galvanic response[s]. Would you therefore agree that in addition to th[e] group termed "vestibular neurectomy" there is [a] group of vestibular disorders where the lesion [is] located in the vestibular receptor organ. The secon[d] observation concerns the use of some term for thes[e] vestibular disorders where there is no involvement o[f] hearing but where there is some doubt as to th[e] location and nature of the lesion. The late Si[r] Terence Cawthorne used the non-committal term "acute vestibular failure" and presumably one coul[d] use the term "chronic vestibular failure" where th[e] vertigo was extended over a longer period of time either continuously or paroxysmally

C. R. Pfaltz (Reply) to Mr *Fisch*. We too have observed a bilateral suppression effect upon the vestibular responses following a unilateral vestibular functional loss. This is due to a specific vestibular mechanism for which we have chosen the term *accommodation*. It is most probably dependent upon the integrity of the efferent vestibular system (inhibition of vestibular responses on the unimpaired side).

To Mr *Hinchcliffe*: If we encounter a case with normal auditory findings and with abnormal caloric response but normal galvanic reaction we may assume the presence of an endorgan lesion. If also the galvanic reaction is reduced or abolished the site of the lesion is at the level of the peripheral neuron. Instead of using the term of "vestibular neuronitis" we prefer the term of "sudden loss of vestibular function" which is less committal, because the pathology of this very characteristic vestibular syndrome is still unknown. In the majority of our cases it is not due to an endorgan lesion but to a retrolabyrinthine disorder of sudden onset, involving sometimes even the vestibular nuclei.

Acta Otolaryng 73 190-196, 1972

RELATIONS ENTRE « PATTERN » ÉLECTROCOCHLÉOGRAPHIQUE ET PATHOLOGIE RÉTRO-LABYRINTHIQUE

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Abstract
Parmi les différentes formes de réponses électro-cochléographiques que l'on peut rencontrer dans la grande variété de situations pathologiques, certaines semblent correspondre plus particulièrement à une pathologie rétro-labyrinthique. (Neurinomes VIII, VII — Ictères Nucléaires — Névrites — Tumeurs cérébrales — Syndromes Neurologiques). Ce sont les réponses *larges* présentant parfois un *pic positif précoce* suivi d un potentiel d action distordu, ou constituées d'une *déflexion brutale négative* qui peut durer plusieurs milli-secondes. Bien qu'il soit difficile de dissocier dans ces images *périphériques*, les éléments purement *sensoriels* des éléments *nerveux* il semble que de telles lésions rétrolabyrinthiques retentissent sur le fonctionnement de l'organe périphérique à ces deux niveaux.
De telles observations chez l'homme et leur comparaison avec les résultats de l'expérimentation animale vont permettre d'une part de préciser le diagnostic dans les cas d'atteintes rétrocochléaires, d'autre part d'approfondir nos connaissances sur les processus qui conditionnent la mise en forme du signal nerveux par le récepteur périphérique.

L électrocochléographie humaine a pu se développer ces dernières années grâce à la méthode décrite par l'un de nous (Aran, 1971 Aran & le Bert, 1968 Aran et al., 1969 Portmann & Aran, 1971 Portmann et al. 1967 1968).

Sur une série de plus de 500 oreilles humaines testées par ce procédé dans notre Laboratoire Clinique nous voudrions rapporter ici, 24 observations dans lesquelles la forme de la réponse s'est révélée nettement anormale.

La réponse étant essentiellement spécifique, toute modification de la forme par rapport à ce qu elle devrait être implique l'idée qu'il existe un disfonctionnement. Il nous a [...] intéressant de faire une étude de la rela[...] qui existait entre les « patterns » obtenus et [...] pathologie du malade.

On peut définir en effet grossièrement ci[...] types de réponses au clic non filtré ou fi[...] par bande de fréquences (Fig. 1)

1 accident négatif d'origine nerveuse, d'au[...] plus étroit que le clic est composé [...] fréquences plus aigues réponse normale
2. réponse diphasique à l'accident négatif [...] succède une réponse assez large positive.
3 réponse dissociée à l'accident N_1 succè[...] une réponse N_2 plus large et plus lente.
4 la réponse est exclusivement négative m[...] beaucoup plus large que la réponse N_1
5 même type de réponse qu en 4 mais p[...] cédée d'un accident très aigu, positif.

Nous avons actuellement suffisamment d'o[...] servations pour affirmer que, pour les fréque[...] ces très aigues ou le clic non filtré, la répon[...] normale doit être de type 1

Lorsqu'il existe une perturbation de la coc[...] lée notamment des cellules sensorielles a[...] recrutement, on trouve habituellement la r[...] ponse diphasique de type 2 auquel s associe[...] d autres symptomes telles la disparition de [...] première phase de la courbe « input-output [...] l augmentation de la raideur de la pente de [...] courbe et enfin, la diminution considérable [...] la latence au seuil

Les réponses 3 sont en général en rappo[...]



Fig 1 Les réponses sont enregistrées au maximum (80 ou 100 dB). Sur cette figure et les suivantes durée des tracés 10 ms, début d'analyse 1 ms avant l'arrivée du clic à l'oreille. Les enregistrements sont tels qu'une déflexion vers le haut correspond à la cochlée positive par rapport au lobe de l'oreille.

avec des surdités de réception exclusives sur les sons aigus. Tout se passe alors comme si l'accident N_2 plus lent correspondait à la stimulation des zones graves de la cochlée tandis que l'accident N_1 n apparaissant que pour un son d'intensité plus élevée et avec une latence beaucoup plus brève, correspondrait à la réponse des fréquences aigues à partir du seuil pathologique.

La réponse 4 n a un caractère normal que si elle suit une stimulation de fréquence grave mais cet élargissement de N_1 est tout à fait anormal lorsqu il se recontre pour un clic filtré aigu ou non filtré.

La réponse 5 est toujours la manifestation d'un trouble pathologique du système nerveux rétro-cochléaire.

C'est l'étude de ces deux derniers types de réponses (4 et 5) anormales, que nous nous proposons de faire à l'occasion de l'analyse de 24 observations, dans lesquelles nous avons trouvé ces « patterns » électrocochléographiques (16 adultes et 8 enfants)

Parmi les adultes, nous citerons

— 7 neurinomes de la VIIIème paire à des stades variés,
— 1 neurinome de la VIIème comprimant le VIII dans le conduit auditif interne,
— 1 tumeur du cervelet opérée avec séquelle de la VIIème paire,
— 1 compression de l'artère vertébrale opérée d'incusectomie,
— 1 névrite grippale,
— 1 sclérose vasculaire,
— 2 surdités brusques d'origine vasculaire,
— 2 cas de vertiges d'origine indéterminée.

Parmi les enfants, on relève

— 4 cas d'ictère nucléaire,
— 1 syndrome de West,
— 2 infirmes moteurs cérébraux,
— 1 origine indéterminée.

Il paraît intéressant de rapporter quelques observations.

Observation N° 1 M. J 25 ans

Histoire clinique Cette malade vient consulter en 1969 pour un syndrome acoustico-facial droit. L'histoire débute à l âge de 14 ans par un hémispasme facial droit. Deux ans plus tard, celui-ci se double d'un début de paralysie faciale du même côté. Aucun diagnostic étiologique n est porté.

D abord incomplète, l'atteinte de la VIIème paire se précise progressivement et une surdité de réception commence à se manifester trois ans après. Enfin, après un laps de temps de six à sept ans, apparaissent des crises de vertiges.

Lorsque la malade est examinée en 1969 elle présente un syndrome vestibulaire de déficit discret du côté droit, une otoscopie normale. La paralysie faciale est toujours complète

Tests complémentaires Oreille gauche audition strictement normale seuil d'intelligibilité 10 dB Oreille droite surdité de réception avec chute progressive sur les aigus. Léger Rinne négatif surajouté au niveau des fréquences graves indépendant de la lésion. Absence de recrutement, Fowler difficile, la malade se plaignant de diplacousie Pas de fatigabilité, mais on remarque que la discrimination est très pauvre (40 %) et ce dernier facteur est donc évocateur d'une atteinte rétro-labyrinthique On trouve neurinome du nerf facial.

Electrocochléographie Pattern anormal de type 5

Observation N° 2 R. S. 30 ans pathologie vertébro-basilaire

Histoire clinique Pas de vertiges, mais acouphènes importants aggravation progressive

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De telles observations chez l'homme et leur comparaison avec les résultats de l'expérimentation animale vont permettre d'une part de préciser le diagnostic dans les cas d'atteintes rétrocochléaires, d'autre part d'approfondir nos connaissances sur les processus qui conditionnent la mise en forme du signal nerveux par le récepteur périphérique

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La réponse étant essentiellement spécifique toute modification de la forme par rapport à ce qu'elle devrait être implique l'idée qu'il existe un disfonctionnement. Il nous a paru intéressant de faire une étude de la relation qui existait entre les « patterns » obtenus et la pathologie du malade.

On peut définir en effet grossièrement cinq types de réponses au clic non filtré ou filtré par bande de fréquences (Fig 1)

1 accident négatif d'origine nerveuse, d'autant plus étroit que le clic est composé de fréquences plus aigues réponse normale.
2. réponse diphasique à l'accident négatif N_1 succède une réponse assez large positive.
3 réponse dissociée à l'accident N_1 succède une réponse N_2 plus large et plus lente.
4 la réponse est exclusivement négative, mais beaucoup plus large que la réponse N_1
5 même type de réponse qu'en 4, mais précédée d'un accident très aigu, positif

Nous avons actuellement suffisamment d'observations pour affirmer que, pour les fréquences très aigues ou le clic non filtré, la réponse normale doit être de type 1

Lorsqu'il existe une perturbation de la cochlée notamment des cellules sensorielles avec recrutement, on trouve habituellement la réponse diphasique de type 2 auquel s'associent d'autres symptomes telles la disparition de la première phase de la courbe « input-output » l'augmentation de la raideur de la pente de la courbe et enfin, la diminution considérable de la latence au seuil.

Les réponses 3 sont en général en rapport

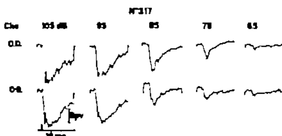


Fig 3 Observation N° 5 réponses au clic à l'oreille droite et l'oreille gauche.

nucléaire — Enfant Rhésus. Exsanguino-transfusion. Placée en couveuse pendant 3 mois.

Tests audiométriques non réalisables.

Electrocochléographie Formes anormales très larges et assez analogues pour les deux oreilles O.D seuil à 60 dB 9 600 seuil à 70 dB, 2 400 seuil à 75 dB O G seuil à 65 dB 9 600 seuil à 80 dB 2 400 seuil à 65 dB (Fig. 3)

Observation N° 6 M. J 56 ans troubles vasculaires de la fosse postérieure

Surdité droite brutale il y a 5 ans avec vertiges et malaises. Surdité brusque gauche d'origine vasculaire récente.

La malade présente par ailleurs un syndrome vasculaire diffus.

Electrocochléographie O G seuil au clic 45 dB, forme large avec pic positif au 9 600 (Type 5). O D pas de réponse au clic à 105 dB (Fig. 4).

Observation N° 7 A. F 3 ans 8 mois

étiologie inconnue

Histoire clinique Les antécédents sont peu connus. Les parents ne sont pas français. La maman a eu vingt grossesses et A. est l avant dernière. Il n'y aurait pas de surdité familiale, ni d'antécédents otitiques.

On note cependant des fistules pré-auriculaires, sans écoulement petit pertuis en avant du tragus. Il y a un an, opération d'une fistule cervicale gauche.

Tests audiométriques L enfant est assez coopérante, la réaction au nom est certaine à 70 dB. Les jouets sonores sont perçus à moyenne intensité (graves et aigus) Le conditionnement avec des cubes est possible et montre Oreille droite une surdité quasi totale. Oreille gauche une surdité partielle avec courbe linéaire aux environs de 70 dB.

Electrocochléographie Alors que pour l'O.D Il est difficile d'observer une réponse même au clic à 105 dB à l'oreille gauche on observe des réponses, mais anormales (Type 5) pic positif et onde lente très négative puisqu elle ne revient pas à zéro au bout de 10 ms (Fig 5) Le seuil est trouvé à 65 dB, le seuil pour le 9 600 à 60 dB.

On notera aussi la forme de la réponse au 9 600 à 90 dB comparable à celle déjà observée dans d autres cas.

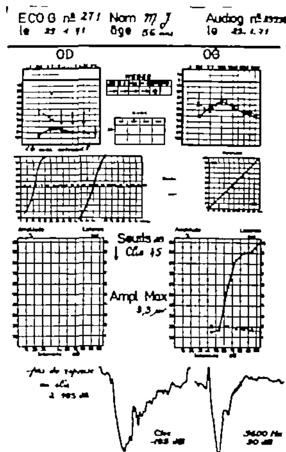


Fig 4 Observation N° 6 fiche rassemblant les résultats des examens audiométriques et électrocochléographiques. Noter les réponses au clic (large) et au clic filtré 9 600 Hz (pic positif précoce).

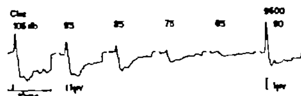


Fig 5 Observation N 7 réponses au clic et au clic filtré à 9 600 Hz. Noter l'importance du pic positif.

En conclusion

S'il existe donc à gauche une audition avec restes auditifs larges autour de 60 dB il est à craindre qu il y ait des difficultés dans le développement du langage, au vu de ces formes anormales qui correspondent à une mauvaise intelligibilité

Etant donné la situation familiale, on envisage le placement dans un Centre de Sourds dès les prochains mois.

DISCUSSION

Devant ces constatations, plusieurs problèmes peuvent se poser

Y-at-il une relation entre ces formes anor ...es et un diagnostic de localisation des ...ubles?

Comment interpréter sur le plan physiopathologique l'apparition de ces formes anormales?

Quelles sont les conséquences cliniques que l'on peut en tirer?

1 *Relation avec un diagnostic de localisation*

Les faits peuvent se résumer de la façon suivante ces formes anormales ne se rencontrent jamais dans les surdités de transmission ou sur des sujets normaux.

De même, on ne les rencontre qu exceptionnellement dans les cas où l'on peut affirmer que la lésion siège exclusivement dans l oreille interne

On les rencontre généralement sur les malades présentant des lésions rétro-labyrinthiques ou dont le syndrome est susceptible d'objectiver une participation du système nerveux.

Il est donc logique de penser que ces formes anormales sont liées à des perturbations du nerf auditif lui-même, pouvant être associées à d'autres troubles cochléaires par exemple ou induits par des lésions plus haut situées (noyaux bulbaires par exemple)

Le caractère rétro-labyrinthique des lésions responsables semble de toutes façons assez bien démontré.

La série d'observation présentée est cependant notoirement insuffisante (7 neurinomes seulement) pour affirmer que l'inverse est vrai, c est-à-dire qu'il n'y a pas de lésion rétrolabyrinthique, même au début de leur évolution sans formes anormales.

Toutefois, la comparaison des groupes étiologiques avec d'autres causes de surdité est également intéressante notamment chez l'enfant les surdités par embryopathie rubéolique ne présentent jamais ces formes anormales, or on sait que cette affection porte essentiellement sur les cellules formatrices du labyrinthe périphérique et non pas des voies nerveuses correspondantes.

2. *Essai d'interprétation physio-pathologique*

Si l'on admet que ces perturbations portent sur le nerf lui-même comment en interpréter la forme?

L élargissement de l'accident N_1 est dû à l'étalement dans le temps des influx nerveux parcourant les différentes fibres après la stimulation périphérique. Il correspond donc à une désynchronisation de ces derniers. Il pourrait s'agir d'une difficulté de conductibilité de certaines fibres du nerf entraînant un délai plus long dans le cheminement des influx.

Il est intéressant de noter que Kupperman (1971) a constaté des formes semblables chez l animal dont les noyaux bulbaires avaient été électro-coagulés.

Peut-être s'agit il également d'une perturbation de la fonction des fibres efférentes comme pourraient le laisser supposer les travaux de Daigneault et al. (1970) qui constatent des

formes anormales analogues après section du faisceau olivo-cochléaire de Rasmussen dans le conduit auditif interne chez le chat.

Quant à l accident positif très bref précédent quelquefois la réponse anormale son temps de latence paraît tellement court qu'il est difficile de l'interpréter comme un accident nerveux. Il s agirait plus probablement d'un phénomène microphonique (différence de temps de latence) entre microphonique positif et négatif ce qui entraînerait l'impossibilité lors de leur neutralisation artificielle, de supprimer ce pic positif

S'il en est ainsi, le trouble proviendrait de l'organe de Corti lui-même. Une étude ultérieure devra être entreprise pour rechercher si la lésion dans ce dernier cas, peut avoir simultanément perturbé organe de Corti et voie nerveuse rétrolabyrinthique.

3 *Intérêt clinique*

Bien que des études complémentaires soient indispensables, l'intérêt clinique paraît dès maintenant certain.

L'apparition de formes anormales (type 4 et 5) lors de l'électrocochléographie incite toujours à poursuivre des investigations radiologiques et neurologiques rétro-labyrinthiques poussées et à déterminer par l'interrogatoire une appréciation plus exacte de l'étiologie qui aurait pu entraîner des perturbations du système nerveux lui-même.

SUMMARY

Among the different patterns of the electro-cochleographic responses that can be observed in the great variety of pathological conditions, some can be related to retro-labyrinthine pathology (VIII and VII neurinoma, Kernicterus, cerebral tumours, neurological syndromes). They are the *broad* responses, presenting sometimes an *early positive peak* followed by a distorted action potential, or comprising an abrupt negative shift of the trace, which can last a few milliseconds. Although it is difficult to dissociate, in these peripheral signals, the parts corresponding to the sensory and/or to the neural structures, it seems that these so-called retrocochlear lesions affect the function of the peripheral receptor at these two levels. Such observations in man, and their comparison with the results of animal experiments, will lead to a refined diagnosis in cases of retrocochlear disorders, and to a better understanding of the genesis of the neural signal in the peripheral receptor

ZUSAMMENFASSUNG

Unter den verschiedenen Arten von elektrocochleographischen Antworten, die man in der grossen Mannigfaltigkeit von pathologischen Situationen antreffen kann, scheinen einige ganz besonders einer retrolabyrinthischen Pathologie entsprechen (Neurinome VIII VII Kernicterus-Nerventzündungen-Gehirntumore-neurologische Syndrome). Das sind die *breiten* Antworten, welche mitunter eine *vorzeitige positive Spitze* gefolgt von einem verrenkten Wirkungspotential beinhalten, oder aus einer *groben negativen Abweichung* die einige Millisekunden dauern kann, gebildet sind. Es ist wohl schwierig unter diesen *peripheren* Gleichnissen die rein *sensorischen* und *nervösen* Elemente zu trennen, es scheint jedoch, dass solche retrolabyrintische Verletzungen auf das Funktionieren des benachbarten Organs in diesen beiden Ebenen einen Einfluss hat. Solchartige Beobachtungen beim Menschen und ihr Vergleich mit den Resultaten der Tierversuche, werden zu einem Teil erlauben die Diagnostik im retrocochlearen Schlagfalle zu präzisieren, zum anderen Teil unsere Kenntnisse über die Prozesse, welche die Formgebung des Nervensignals mit dem benachbarten Empfänger entscheidend beeinflussen, zu vertiefen.

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DISCUSSION

J E. Bordley I enjoyed very much Mr Portmann's presentation. I am sure that this technique offers much for further studies of deafness. I would like to cite three things that were recorded in our laboratory some years ago, when we were studying human cochlear potentials. These studies were carried out by Ruben, Sekula, Fisch, Bordley et al.

1 A child aged 7 years suffering from Kernicterus and with subjective severe deafness gave normal cochlear potentials.

2. In cats when the VIII nerve was sectioned by Bard's technique, using gentle section rather than sharp dissection in order to preserve the blood supply we obtained normal cochlear potentials.

3 After section of the olivo-cochlear bundle in a cat. The response increased and the point of over d was greatly increased but the tracing (N1 and N2) normal. We did not have the advantage of a summation computer in our studies; our observations were made on series of individual recordings.

J Groen. Mr Portmann! First I want to congratulate the School of Bordeaux on their experiments which we tried to imitate: the best flattery is imitation. Among the parameters you discussed here the amplitude as a function of time, and latency My question is: did you find anything abnormal in the latency in the group of I believe, 25 patients, you discussed in the later part of your presentation?

G Lidén. I have enjoyed Mr Portmann's presentation very much. I should like you to comment further on the possibilities to use electro-cochleography for diagnosing early cases of retrocochlear lesions. It would also be of great interest for future work if you could correlate the results of electrocochleography with the results of classical and some newer audiological tests such as impedance audiometry and sound localization test.

M Portmann (Réponse). Je remercie Mr Bordley pour ses commentaires. Il a une grande expérience de ces problèmes dans sa clinique. No. 1 I have the same experience. We found in Kernicterus some cases without "retro cochlear" E.Co.G abnormal pattern. The variation of the pathology can explain this fact. Nos. 2 and 3 These two experimental results on animals are very interesting. We must accumulate this sort of data in the future both on human pathology and on animal experiments to try to explain the pathogenesis of this kind of pattern.

The latency was not significantly modified in this series of 25 patients with abnormal pattern responses.

We don't know yet if this method is more efficient to diagnose the very early stage of VIII nerve tumour. More data are necessary to be sure that it is a better approach than the classical audiology

Acta Otolaryng 73 197–202, 1972

CORTICAL AUDIOMETRY

The Influence of Stimulus Intensity upon Evoked Auditory Potential in the Normal Person and in Cases of Hearing Loss with Recruitment

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Abstract. The author demonstrates how there is a direct and proportional relation between the intensity of the stimulus and the amplitude of the E.R.A. for people with normal hearing as well as for patients with transmission or reception hearing loss. However when the stimuli are intense in people with normal hearing the amplitude of the E.R.A. diminishes, and in patients who suffer a hearing loss with recruitment, the E.R.A. reaches a greater amplitude than the intensity of the sonorous stimulus used.

There is a relationship between the characteristics of sound as an auditory stimulus, and the auditory sensation produced by this stimulus. Depending upon the frequency of the sound used as a stimulus, the sound produced will be of a high-pitched, bass or medium tone Thus the clarity of the sensation produced, in relation to the frequency of the auditory stimulus, will depend upon the clarity of this stimulus, which is the basis or physical characteristic of the tone.

With reference to the intensity the intimate and direct relationship which exists in normal hearing between the intensity of the auditory stimulus and the clarity of the perceived sensation which it produces, is a well known fact. We therefore classify sounds on the basis of this relationship into weak or slightly intense medium, and strong or very intense.

Until recently in audiology we could not objectify so to speak, the relation between the sonorous intensity perceived and the extent of the vibration which produces it. And so to measure the intensity we created distinct units, of which the best known and most used is the decibel.

With the introduction into audiology of the examination of evoked auditory potential (E.R.A.), it is possible to state, with the extrapolation it implies, that we have found the conditions which enable us to establish an objective relationship between the physical basis of the intensity or the extent of the vibratory movement which produces it, and the sensation produced or evoked by it, whether weak, medium or strong. From electroencephalographic examinations of the E.R.A. we know that there exists, in general, a direct and proportional relationship between the intensity of the auditory stimulus and the extent of the components of the evoked auditory potential. This we have confirmed in our examinations.

It is a known fact that in people with normal hearing, as well as those with conductive hearing loss, and others having a damaged cochlear nerve, a linear relationship exists between the sonorous stimulus and the auditory sensation. This is produced in such a way that when the intensity of the stimulus increases, the auditory sensation does likewise and when the sonorous intensity diminishes, the perceived sonorous sensation is less.

However in certain types of deafness, this linear relationship is lost or disappears, as in

cases of hearing failure due to damage at the point of the sensory receptors. Then various phenomena of distortion arise, and one may observe the intensity distortion which is of great value for the topographic diagnosis of deafness. This occurrence is probed in clinics by investigating the recruitment phenomena.

All the audiometric techniques which try to demonstrate whether or not recruitment exists in the presence of definite deafness, have a common defect, i.e. subjectivity which makes it difficult or even impossible in many cases to accomplish properly the necessary investigations or evaluate the results. This occurs, for example, with children, old people, the mentally ill, or simulators.

It is of great importance in the diagnosis of Menière's disease to know if recruitment exists. That is to say there is a distortion of the intensity function which means the same as the loss of the linear relationship between the intensity of the auditory stimulus and loudness perceived. The subjectivity of the procedures utilized for this investigation have been done by us by means of the examination of the evoked auditory potential or cortical audiometry in patients with Menière's disease. The data being furnished by tonal and vocal audiometry Fowler's test, Lüscher SISI automatic audiometry and tone decay test, confirmed the existence of an auditive defect with the characteristics of reception hearing loss.

Of the 21 patients examined, 11 were men and 10 were women. Ages ranged from 26 to 51 years. Evolution of the disease was between 7 months and 4 years. There was a variable degree of hearing loss, the auditive deficiency being greater in those patients with a longer evolution of the disease.

The investigation was done in a silent room using a Medelac Amplaid apparatus incorporating an electronic computer with the following technical arrangements: the active electrode was placed at the vertex, the ground electrode on the forehead, and a reference electrode on the mastoid. The stimulus frequencies were 500 1 000 2 000 and 4 000 cps. In the last three tests it was possible to observe the recruitment. The test consisted of 20-30 stimuli and included 10 whose results were very clear. The duration of each tone pulse was 300 msec. The rise and decay time was 25 msec and the tone pulse was presented at a rate of one every 2 sec. The sensitivity of the apparatus was 7–15 mV

It is known that intensity is a fundamental characteristic of the auditory stimulus necessary for an exact evaluation of the E.R.A. This is due to the intimate relationship which exists between the intensity of the sonorous stimulus used and the amplitude of the evoked response. Thus when there is a greater intensity of the auditory stimulus there is a greater range in the waves or E.R.A., and at a lesser intensity there is a smaller range. Therefore there is a directly proportional relationship between both phenomena. One concerns the physical, the sonorous intensity and the other concerns the neurophysiological, i.e. the amplitude of the evoked cortical response.

On the other hand, there is also a close relationship between the intensity of the stimulus and the latent period of the cortical potential. But in this case the relationship is inversely proportional, so that at a greater intensity the latent period is shorter and at lesser intensity the latent period is longer

This double relationship between the sonorous intensity the extension, and the latent period, is fundamental for planning the auditory threshold during the E.R.A. examination. This will avoid a false interpretation and artificial techniques.

What stands out is that in subjects with normal hearing (except under intense stimuli) and in those with transmission hearing loss, as well as those with damaged cochlear nucleus and central auditory pathways, a direct and proportional relationship is, in general, maintained between the intensity of the auditory stimulus and the extension of the E.R.A. When the sonorous intensity is greater so is the E.R.A. amplitude, and vice versa. In cochlear deafness the relation with stimuli of

high intensity is lost. We were able to evaluate this during observation with audioelectroen cephalography or cortical audiometry which we shall now describe.

During the investigation of damaged hearing, one uses stimuli of low intensity and then medium intensity while the extension of the waves (or units of E.R.A.) increase in linear form The relationship is directly proportional to the intensity However if we continue increasing the intensity of the auditory stimuli until we reach high levels, the E.R.A. extension then increases in an abnormal manner This is clear when compared with what occurs in normal hearing, or in those affected by transmission or retrocochlear hearing failure.

This functional alteration must be attributed to the commencement of a distortion phenomenon in the performance of the intensity This is precisely what happens, and almost exclusively in cases of cochlear hearing failure. One must admit that when sensory receptor elements are stimulated by high intensity sounds, they respond in an abnormal way sending false messages to the centres. Regarding the performance of intensity as expressed at the cortex level, the E.R.A. registers an exaggerated range in relation to the intensity of the stimulus used and, subjectively in tolerance is registered in many cases to the sonorous stimulus.

Davis & Zerlin (1966) and Picton et al. (1970), claim that in the normal person they do not find the linear relationship which exists between auditory stimulus intensity and the extension of the E.R.A. for low and medium intensity so that the relationship for high intensity stimuli is lost. Picton et al. (1970) state that when the intensity of the stimulus is more than 70 dB the E.R.A. amplitude tends to diminish, causing a levelling in the waves registered.

We also have observed the loss of this relationship under high intensity stimuli, in people with normal hearing. This occurs at levels of varying intensity with each individual

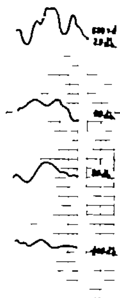


Fig 1 Normal hearing person. Observe how the amplitude of the E.R.A. diminishes when the intensity of stimulus increases.

and it occurs in such a way that with some people the diminishing of the extension of the E.R.A. begins to appear when the stimulus reaches 60 dB In others a stimulus of 80–100 dB of sonorous intensity is required.

We believe this data is extremely interesting, and we wish to state that the diminution of the E.R.A. amplitude in normal people under observation when using high intensity auditory stimuli, or the levelling and near disappearance of the units of evoked cortical potential, do not share the corresponding diminution of the sonorous sensation. At these levels there is a loss of the direct and proportional relationship between the amplitude of the E.R.A. and the sonorous sensation perceived.

We have observed in the outline of the E.R.A. in normal people (Fig. 1) with high intensity auditory stimulus, a completely opposite fact to that which occurs in people affected with hearing loss with recruitment (Fig. 2) as occurs in Menière's disease. With the latter the intense stimulus provoked an exag

mindert sich das Ausmass des E.R.A., und erreicht in den Personen, die unter Hypoacusie mit Recruitment leiden, ein grösseres Ausmass als die Intensität des angewandten sonoren Reizes.

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DISCUSSION

R. Hinchcliffe Peux je dire que mes collègues, Messrs Beagley et Knight ont demontrés ce phénomène de recruitment auditive avec les potentiels evoquées cérébraux. Ils ont rapportés leur resultats au IX° Congrès de la Société Internationale d'Audiologie à Londres 1968.

J Marco (Réponse) à Mr *Hinchcliffe* Je suis d'accord que M. Knight et M. Beagley ont trouvé resultats très resemblables avec ceux de moi-même.

Acta Otolaryng 73 203–211 1972

NEONATAL CHANGES IN THE ENDOLYMPH AND THEIR POSSIBLE RELATIONSHIP TO PERI NATAL DEAFNESS

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Abstract. The electrochemical changes in the cochlear endolymph were investigated in rats aged 8 to 18 days. The endocochlear potential was very small until the 12th day when a 50 mV increase occurred in 48 hours, but the ionic concentrations were already mature on the earliest day studied. The extremely rapid potential increase appears to be associated with fundamental membrane changes and the cochlea thus seems to pass through a critical and potentially hazardous developmental phase at this time. Such a phase seems important in the pathogenesis of genetically-determined deafness in animals but may be less so in man. However due to its early maturation, the human cochlea is uniquely vulnerable to peri-natal anoxia and this may initiate degeneration in the presence of other deleterious factors.

Perhaps the most important and certainly one of the most striking aspects of genetically-determined deafness in animals has been the discovery that the inner ear continued to develop normally until shortly after birth. The pathological changes are thus attributable not simply to a failure of cochlear development, but to some degenerative condition whose onset is delayed until maturation is fully or almost fully completed. This notable feature was first reported in the mouse as long ago as 1906 by Quix, whose histological observation was soon confirmed and extended, not only by the morphological studies of his pupil van Lennep (1910) but also by the behavioural findings of Yerkes (1907)

Since then many similar light microscopic and gross functional studies have revealed the same general pattern of events to occur in a large number of mutants of this type, both in mice and in other laboratory mammals In recent years, with the wider use of the electron microscope and the development of sophisticated electrophysiological methods of investigation, the ability to detect pathological changes earlier in development clearly existed.

Hilding and his co-workers (Kikuchi & Hilding, 1965 *b* 1967 Hilding et al., 1967) in their very able anatomical investigations were, nevertheless, unable to demonstrate any abnormality in cochlear development until the second week after birth in both the sh-1 mouse and the Hedlund white mink. On the other hand, Mikaelian & Ruben s (1964) careful investigation of the initiation of the responses to sound in the sh-1 mouse showed these to be all slightly delayed, the cochlear microphonic potentials, in particular appearing typically on the 9th instead of the 8th day after birth. Both the range and magnitude of the cochlear microphonic and action potentials never reached normal levels and eventually regressed, completely disappearing by the end of the third week. The onset of the pathological process, therefore, seemed to coincide with the period after birth associated with the inception of hearing.

These studies undoubtedly confirmed that the onset of the cochlear degeneration was indeed delayed until the postnatal period The possible significance of this finding was first emphasized by Grüneberg et al. (1940) who suggested some adverse environmental circum

stance might, in consequence play a possible role in the aetiology of the deafness.

Bosher & Hallpike (1965) were able to take this matter much further in their investigation of the pathogenesis of the inner ear degeneration in the deaf white cat. Of fundamental importance was their finding that the pene trance of the deafness was only 80% In other words, every fifth labyrinth escaped degenera tion even though the individual was known to possess the gene because of the presence of a white coat colour or occasionally because of degeneration in the other ear

This clearly established that, although the gene concerned was primarily responsible for the inner ear deafness, some other factor was essential for the initiation of this process, i.e for enabling the genetic defect to exert its damaging action. The nature of this additional factor is obviously of consequence and here Bosher & Hallpike were able to prove that it was not genetic and so must be environmental in origin.

Little of certainty about its effect could be deduced from their entirely histological observations, but from certain features of the pathological process, these authors thought its might be limited to some particular phase in the maturation process of the endolymph system where it was capable of produc ing some abnormality in the endolymph In the remarkably similar deafness in the Hedlund white mink, Hilding and his co-workers (1967) reached much the same conclusion for in these animals the first ultrastructural pathological changes appeared in the stria vascularis.

The identity of the possible aetiological agents concerned still remains obscure as does the nature of the endolymphatic homeostatic mechanisms themselves. However the unique high potassium, low sodium composition of the mammalian endolymph, together with the endocochlear potential is now known to be maintained entirely by active mechanisms, which are highly oxygen dependent (Bosher & Warren, 1968 Konishi & Mendelsohn, 1970 Kuijpers & Bonting, 1970) It consequently seemed worthwhile to investigate whet electro-chemical changes occurred in the in ear fluids at the time of the inception hearing, since this appeared, from the worl Mikaelian & Ruben (1964) to be one of periods, at least in animals, when the in ear is at risk.

MATERIAL AND METHODS

The experiments were conducted on w Wistar rats, whose age was estimated from time of conception to avoid the unaccepti variation in the results due to differences in length of gestation, when the age was rel to the date of birth For comparison with findings of other workers, the postnatal has been calculated assuming a gestation pe of 22 days (the average in the stock used).

The animals were anaesthetized with thane (0 01 g/10 g body weight by int peritoneal injection) and, after the tympa cavity had been exposed by means of a late approach a small fenestra was made in labyrinthine capsule over the stria vasculari the middle turn. The endocochlear poten was next measured using a Pyrex micro-el trode tip diameter 1 μ filled with a fl comparable in composition to endolymph, then a Pyrex micropipette, tip diameter 5 was inserted at the same site and to the sa depth as the micro-electrode to enable quantity of endolymph, limited to about 20 to be aspirated. A further measurement of endocochlear potential was subsequently m and, if any significant alteration had curred from the original value the experim was terminated, since undesirable structu damage was considered to have taken pla Finally a specimen of perilymph was obtai from the adjacent scala vestibuli of the ba turn.

A digital voltmeter (Solartron L 1604) used in conjunction with an electrometer p amplifier (Analogue Devices 310) to meas the endocochlear potential. The sodium a potassium concentrations of the inner

fluids were estimated by means of total emission flame spectrophotometry (Bosher & Warren, 1968) and an electrometric technique was utilised for the estimation of the chloride concentrations (Ramsay et al., 1955) Where necessary the osmotic pressure of the fluids was determined by means of the freezing point depression method of Ramsay & Brown (1955). Unfortunately in many instances the volume of endolymph which could be obtained was insufficient for a full range of analyses to be undertaken.

A full description of the technical methods, including details of the elaborate precautions necessary to prevent contamination, partic ularly of the endolymph samples, will be found in another publication (Bosher & Warren, 1971), which also contains a comprehensive record and full analysis of the results obtained. Only those findings relevant to the present discussion will be presented here

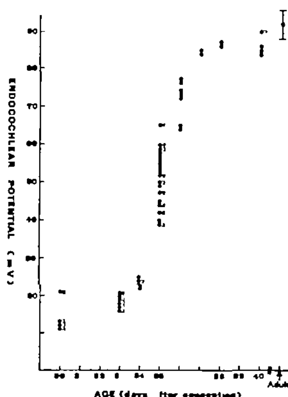


Fig. 1 The change in the endocochlear potential with age. The range and mean of the potential in adult rats is also shown. Numerals indicate the number of experiments giving the same individual result. The a erage length of gestation was 22 days.

RESULTS

Particularly marked changes occurred in the magnitude of the endocochlear potential dur ing the period studied (Fig. 1) On the 8th day after birth the average potential was merely 14 mV and the rate of increase was so slow that 3 days later the mean potential was still only 19 mV But, thereafter it increased surprisingly rapidly particularly between the 12th and 14th day when there was a rise of about 50 mV in 48 hrs. Moreover this phase of rapid change took place practically simultaneously throughout the whole cochlea, since on the 13th day the average potential dif ference between each turn was about 7 m\ a figure not statistically different from the value of 5 mV found to be present in adult animals.

The mechanisms responsible for the sudden rise in the potential remain obscure, but, on the whole, the most likely explanation seems to be that the full potential becomes manifest as a result of a correspondingly rapid increase in the electrical resistance of some portion of the cochlear duct membranes at this time. Cer tainly there can be little doubt this is a period of great change in the endolymphatic system, whatever the process concerned

However the analytical results (Fig. 2) presented a quite different situation. Even on the first day studied, when the potential was still very low the endolymph had virtually attained its adult composition, and, despite the subsequent marked potential changes, remained remarkably constant in this respect.

The reason for this relatively early development and strict maintenance of the mature ionic constitution a matter clearly of import ance, cannot be determined accurately because of the paucity of the evidence available Never theless, it is known that the hair cells appear fully mature, even at the electron mk ᴗ

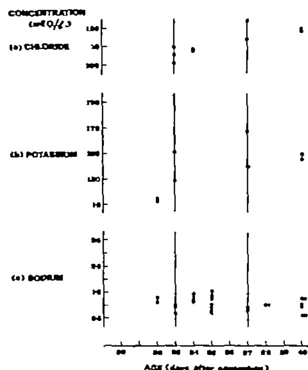


Fig The endolymphatic analytical results. The horizontal lines define the normal adult range for sodium and potassium, the difference in scale will be noted. The vertical lines delimit the total period of rapid development of the endocochlear potential. Numerals indicate the number of experiments giving the same individual result. The average length of gestation was 22 days.

level, before the cochlear microphonic responses develop (Kikuchi & Hilding, 1965 *a*) and also that an increased endolymphatic sodium concentration produces depression of the microphonic responses (Konishi et al., 1966) and even degeneration of both the hair cells and stria vascularis (Duvall & Rhodes, 1967 Duvall, 1968) This early development of the adult endolymphatic chemical composition, it thus seems reasonable to suggest, may be concerned with the integrity of the coincidently maturing hair cells.

Detailed examination of Fig. 2 will reveal both the endolymphatic potassium and chloride concentrations to be a little lower than the usual adult range and, at first sight, the presence of some slight immaturity of the homeostatic mechanisms concerned seems likely On the other hand, the perilymphatic analytical results (Fig. 3) showed a similar situation was present in this fluid too and so this raised the possibility that these somewhat low ionic concentrations resulted not from immaturity but from diminution in the osmotic pressure with dilution of the inner ear fluids.

Further investigation therefore seemed necessary and this did, in fact, confirm that the rather low values found were due merely to some general body hypo-osmolality at this age In 13-day-old animals the range of the osmotic pressures in the endolymph was 7.01–7 40 in the perilymph 7 04–7.32, and in the blood 6.91–7 44 while in adult animals the corresponding range for the blood was 7.12–8 07 (atm at 38 C) Such a confirmation of the interdependence of the inner ear and general body fluid osmolalities has far-reaching consequences, for it implies that some portion, at least, of the membranes bounding the endolymphatic space must be freely or relatively freely permeable to water

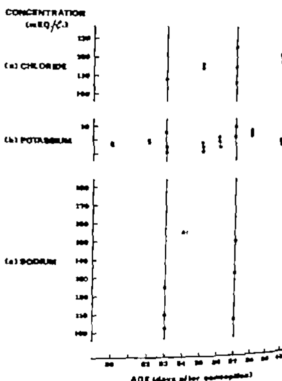


Fig 3 The perilymphatic analytical results. The details are as described for Fig. 2; the differences in scale will be noted.

Consequently an attempt was made to explore this aspect more directly by determining the effects of changes in extracellular osmolality induced by intraperitoneal injection of either distilled water or hypertonic glycerol solutions. In each instance an initial rapid alteration was produced in the osmolality of the endolymph and perilymph, which corresponded both qualitatively and quantitatively to the extracellular changes (for an example see Fig. 4) Although this demonstration of a relatively high water permeability is of significance in other fields, it will not be discussed further here, as a feature of more relevance in the present context was the occurrence of a corresponding change in the chemical composition of the inner ear fluids due to these alterations in the general body fluids.

Finally it should be noted, the large osmotic water movements across the membranes concerned were not associated with any changes in the endocochlear potential. Hence, the electrical leakage in the younger animals studied is almost certainly not accompanied by any major intercellular *ionic shunts*.

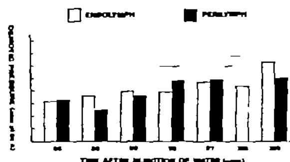


Fig. 4 The changes in the osmotic pressures of the inner ear fluids in 35 day (post-conception) animals after intraperitoneal injection of 1 ml water/10 g body wt. (the time intervals are not drawn to scale). The dotted lines indicate the range found in control animals. The differences between the two fluids in any individual animal are statistically not significant.

DISCUSSION

The general situation revealed by our observations, therefore is that the production of the endocochlear potential largely takes place independently some time after the attainment of the mature endolymphatic chemical composition. Ample evidence has now accrued which reveals the inception of hearing, as judged by the appearance of cochlear microphonic potentials and of action potentials in both the primary and secondary auditory neurones, together with reflex and behavioural responses to sound stimulation, to coincide in a number of laboratory mammals (including the rat) with this period during which the endocochlear potential develops (Schmidt & Fernández, 1963 Änggård, 1965 Kikuchi & Hilding, 1965 Mikaelian & Ruben 1965 Crowley & Hepp-Reymond, 1966)

The period clearly constitutes a very important phase in cochlear maturation which the present investigation has revealed is associated with fundamental changes in some portion, at least, of the cochlear duct membranes, as described above. The discovery in recent years that abnormal genes can produce their effects through structural defects, such as alteration in membrane characteristics (Milne, 1966), thus seems of particular relevance.

As other changes may also occur at this time in the endolymphatic homeostatic mechanism and the hair cell transduction processes, such a period in the functional development of the cochlea must surely be regarded as a critical one During it the ability of the inner ear to counteract the possibly adverse effects of external changes may well be reduced and this phase of development consequently become a potentially hazardous one Indeed, the results reported earlier do provide some evidence in support of this concept, for the homeostatic mechanisms were demonstrated to be unable to prevent alterations in the chemical composition of the endolymph following changes in extracellular fluid osmolality In this case, these were induced experimentally but they might have been the

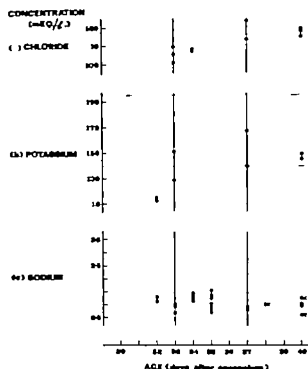


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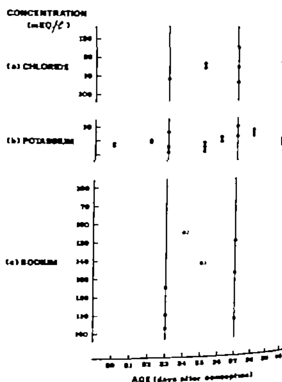


Fig. 3. The perilymphatic analytical results. The details are as described for Fig. 2, the differences in scale will be noted.

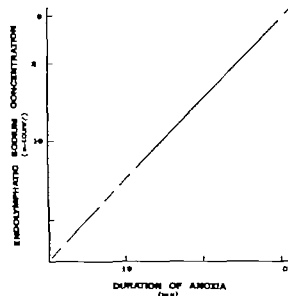


Fig 5 The change in the endolymphatic sodium concentration during anoxia. (From the results of Bosher & Warren, 1968.)

justifiably considered a critical one, during which the human inner ear as a direct result of its unique maturity is indeed likely to be more vulnerable to adverse circumstances.

Certainly the biochemical investigation of the nature and complex interaction of the various factors concerned appears to have a major role in the elucidation of the pathogenesis of human congenital deafness, both in genetically-determined cases and in the large group whose aetiology is as yet ill-understood. Moreover such investigation, by giving a clearer understanding of the likely nature of the defect responsible, should also enable further studies at the cellular level, by such sophisticated techniques as those described by Thalmann (1972) to be undertaken with the precision they require

ACKNOWLEDGMENT

Grateful acknowledgement is made to the Editorial Board of the Journal of Physiology for permission to reproduce Figs 1-4

RÉSUMÉ

Les changements électrochimiques de l'endolymphe cochléaire ont été étudiés chez des rats âgés de 8 à 18 jours. Le potentiel endocochléaire était très petit jusqu'au 12ème jour quand une augmentation de 50 mV se produisit en 48 h., mais les concentrations ioniques étaient déjà mûres au début de l'étude. L'augmentation extrêmement rapide du potentiel semble être associée aux changements fondamentaux des membranes et le limaçon semble donc en ce moment, passer par une phase critique et potentiellement hasardeuse de développement. Une telle phase semble importante dans la pathologie de surdité déterminée génétiquement chez les animaux mais peut être moindre chez l'homme. Cependant, à cause de sa maturité prématurée, le limaçon humain est uniquement vulnérable à l'anoxie périnatale et ceci peut initier la dégénération en présence d'autres facteurs délétères.

ZUSAMMENFASSUNG

Die elektro-chemischen Veränderungen in der cochlearen Endolymphe wurden in 8 bis 18 Tage alten Ratten untersucht. Bis zum zwölften Tage war das endocochleare Potential sehr klein, dann nahm es binnen 48 Stunden um 50 mV zu. Die Ionen-Konzentrationen waren aber bereits am ersten Untersuchungstage reif. Der besonders rapide Potentialanstieg scheint mit grundlegenden Membranveränderungen zusammenzuhängen und die Cochlea scheint demnach zu diesem Zeitpunkt ein kritisches und möglicherweise gefahrvolles Entwicklungsstadium durchzumachen. Ein solches Stadium scheint in der Pathogenese genetisch-bedingter Taubheit bei Tieren von Bedeutung, jedoch bei Menschen nicht so bedeutend zu sein. Durch ihre frühe Reife neigt aber einzigartig die menschliche Cochlea zum perinatalen Sauerstoffmangel und dadurch mag bei Vorhandensein anderer schädlicher Faktoren die Entartung eingeleitet werden.

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DISCUSSION

M Lawrence I admire this work very much, measuring the ionic content of the endolymph of these very small animals. Along with the developing sensory structure there is, of course the developing microvasculature, so is it not possible that the injury to the stria vascularis, even though samples with red cells are eliminated could not be responsible for the changes? Do you have any reservations about these measures in view of the injury that must occur to the developing microcirculation within the stria vascularis

J Angell James Can you say how long was the time lapse after the onset of anoxia before the ionic changes were seen in the endolymph Secondly how much time elapsed after the anoxic period before recovery occurred? Also what was the relation to the biological effects of ultrasound on the inner ear in the treatment of patients suffering from Menière's disease I have noticed a greater tendency to hearing loss after ultrasonic treatment if it was performed under general anaesthesia rather than under local anaesthesia. Taylor of Cogs Hospital had shown that the destructive effect of ultrasound on nervous tissue was markedly increased during anoxic conditions.

R Hinchcliffe Have you attempted to relate the changes that you have observed in the ear to the other biochemical and electrochemical changes that occur in morphosis during this particular two-week period, e.g. the demonstration by Lagerspetz (1962)

that this is the period of development of homothermia. Furthermore, do you think that the ultrasound the neo-natal myomorpha produce during this period is perceived by these young animals?

J J Groen I am particularly grateful to Mr Bosher for mentioning Professor Quix as one of the first to do this kind of experiment. In his last slide I observed a straight line. Looking closely I believe I saw that the ordinate was logarithmic. This suggests that the problem of anoxia as a function of time is merely one of diffusion or depletion. Is the answer to this problem that simple?

E Borghesan During fetal life there is a reabsorption of mesenchymal tissue and consequently an increase of the venous circulation in the modiolus. This hyperaemic phase is followed by a hematic stasis due to the reduction of the vessels. I wish to know whether such stasis and the relative hypoxia may be the cause of the variations observed in the cochlear potentials.

S K Bosher (Reply) to Mr *Lawrence* In the initial stages of our work we, too, were anxious about the possible adverse effects of damage both to the capillaries and also the epithelial cells. However a quite extensive histological investigation revealed the incidence of such damage to be insignificant provided the pipette size was limited as specified and we also found that capillary damage in particular could be easily recognized as it led to gross contamination of the specimen with blood.

To Mr *Angell James* The occurrence of an increase in the damaging effects of ultrasound in the presence of slight anoxia, of which I was not aware, is extremely interesting as it resembles in a general way the situation I believe to exist in the ear. This matter of the time course of the changes in the inner ear fluids in anoxia is, of course, of fundamental importance. There is no doubt that the chemical changes commence immediately the stria vascularis is inactivated by the anoxia, and, interestingly the ionic constitution is rapidly restored when the anoxia is relieved, provided the damage is not irreversible. This suggests to me that normally the stria vascularis possesses great functional reserves. However slight degrees of anoxia, it should be remembered, seem to have little effect upon strial function, although this rapidly declines when a critical level is reached.

To Mr *Hinchcliffe* The rat is very immature indeed during the early portion of the period studied and the electro-physiological investigations previously performed seem to exclude the possibility of any hearing being present before the period I have indicated. On the other hand your comment that great changes are taking place throughout the whole animal is most pertinent but I am afraid their relation to the inner ear can only be a matter of speculation at present.

To Mr *Groen* Your comment is an extremely accurate one for the changes in the ionic concentrations during anoxia are undoubtedly due to the diffusion of the ions concerned down their electrochemical gradients.

To Mr *Borghesan* As I have no personal experience relating to this period of development, I am afraid I can offer no further comment.

Acta Otolaryng 73 212–217 1972

EXPERIMENTAL VASOMOTOR RHINITIS

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Abstract Unilateral and bilateral experimental vasomotor rhinitis was produced in 4 dogs with cervical sympathectomy unilaterally and bilaterally We studied this problem from several points of view in order to explain the mechanism of vasomotor rhinitis and the relationship between upper and lower respiratory tract.

Vasomotor rhinitis may be caused by exaggerated response to air-borne allergens, changes in weather dysfunction of the autonomic nervous system, or extreme mental upset (Ballenger 1969) it may also be caused by surgical interruption of the sympathetic innervation of the nasal cavity In 1943 Fowler described ilateral vasomotor rhinitis in a patient after ...on of the left stellate ganglion Concluding his review he noted, "It is possible that the vasomotor changes in the nose occur or are only permanent with a lesion of a stellate ganglion. Experimental surgery might uncover the fundamental mechanism of vasomotor rhinitis." Later Krajina & Poljak (1961) observed vasomotor rhinitis in several patients following extirpation of the stellate ganglion and studied the changes occurring in the nasal mucosa after cervical sympathectomy in the rabbit.

The arteries, arterioles, and veins of the nasal mucosa are surrounded by a rich plexus of adrenergic nerve terminals. This rich plexus of adrenergic fibers surrounding the wide veins of the erectile tissue in the inferior concha is considered to be of great importance in regulating the blood flow through the nasal mucosa and thereby airflow and heat exchange In contrast to the sparse adrenergic innervation of the veins in skeletal muscle, and in most other tissues studied, the veins within the nasal mucosa are surrounded by very rich adrenergic plexuses (Dahlström & Fuxe, 1965)

The present work describes some effects of surgically induced vasomotor rhinitis on the nasal mucosa and on pulmonary resistance, and some systemic reactions to physical stimulation of the denervated mucosa.

MATERIAL AND METHODS

Experimental subjects were 4 adult mongrel dogs weighing between 15 and 20 kg. Under sodium pentobarbital anesthesia, separate oropulmonary and nasopulmonary resistance measurements were made preoperatively according to the method of Ogura (Ogura et al., 1964 Ohnishi & Ogura, 1969) Vasomotor rhinitis was provoked experimentally by surgical extirpation of the superior cervical sympathetic ganglion the cervical trunk was transected between the first and second ganglia and the carotid artery was stripped This procedure was performed unilaterally in two animals and bilaterally in the remaining two. It should be noted in passing that the superior cervical sympathetic ganglion can be readily

Supported in part by NIH Grant NS 06200 and International Research Board (IREX).

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Fig 1 The anterior nasal mucosa pale and swollen after bilateral cervical sympathectomy

identified by tracing the vagus nerve to its nodose ganglion and then tracing the small but consistent connection to the superior sympathetic ganglion.

Three days after sympathectomy oropulmonary and nasopulmonary resistance measurements were repeated On the fourth post sympathectomy day electromyographic tracings (EMG) were obtained from the intercostal muscles, using unipolar needle electrodes (Nihon-Koden) and a Grass model 7 polygraph. A cold air douche was applied to the nasal air way by means of a Dundas Grant ethyl chloride apparatus two applications of 5 sec each being employed. Intercostal EMG tracings were obtained after each cold air application. Nasal smears for eosinophil counts were obtained bilaterally at intervals from the second through the sixth postsympathectomy days in each animal Biopsy specimens of nasal mucosa were obtained before and some days after sympathectomy for histologic comparison. The sympathetic ganglia and nerves removed at surgery were likewise examined

RESULTS

Sympathectomy resulted in increased irritability of the nasal mucosa and the expected findings of Horner s syndrome in all animals. These observations are recorded here only in passing inasmuch as they are not pertinent to the present experiment. Gross observations in sympathectomized dogs revealed evidence of increased nasal obstruction, including noisy breathing and snorting. The anterior nasal mucosa was visibly pale and swollen (Fig 1) Dilatation of mucosal vessels and hypersecretion of nasal glands were also evident. However the dusky blue color usually seen in humans with vasomotor rhinitis was not noted in our experimental animals.

Electromyographic recordings obtained from the intercostal muscles during and after cold

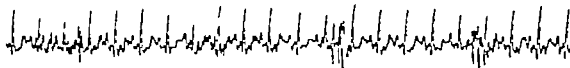


Fig 2 Preoperative electromyogram



Fig 3 Electromyogram 5 days after bilateral cervical sympathectomy

air stimulation of the nostrils showed much greater activity after stimulation of the sympathectomized nostril than of the normally innervated side. In those animals subjected to bilateral sympathectomy the response was even more pronounced (Figs. 2, 3 4) In addition to increased EMG activity the cold air douche produced sneezing when blown into the denervated nostrils. A review of the literature indicated that such a response has not yet been reported experimentally Another finding was the production of glottic spasm after stimulation of the sympathectomized nostril in one imal, and the demise of another animal, bilaterally sympathectomized, produced by the same amount of anesthesia that had been well tolerated preoperatively Histologic examinations of specimens of nasal mucosa taken before and after the operative procedure revealed marked edema, vasodilatation and an increase in mucous gland content in specimens from the operated side (Figs. 5 6) All postoperative nasal smears revealed an increase in the number of epithelial cells. A few eosinophils were found in one smear but no mast cells. Nasopulmonary and oropulmonary resistance was increased in dogs with unilateral vasomotor rhinitis the differences were more pronounced with bilateral vasomotor rhinitis (Table I)

DISCUSSION

Extirpation of the superior cervical sympathetic ganglion produces a vasomotor rhinitis in the dog which is basically similar to that reported in man and rabbits (Krajina & Poljak 1961) Histologic study of the nasal

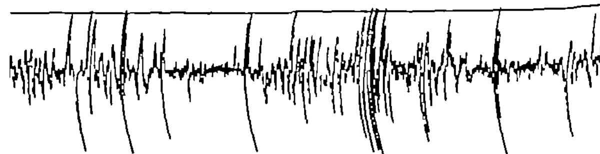


Fig 4 Electromyogram during sneezing caused by blowing cold air into right nostril (site of vasomotor rhinitis).



Fig. 5. Nasal mucosa of the dog before cervical sympathectomy

mucosa and secretions showed no resemblance to the condition caused by allergic rhinitis Pulmonary resistance was increased following sympathectomy and was greater following a bilateral procedure Krajina & Poljak (1961) reported before that the denervated nasal mucosa reacts more strongly to different external stimuli than does normally innervated mucosa. That is true only in the true parasympathicommetic state of the nasal mucosa which is produced by sympathetic denervation. On the other hand vidian nerve resection usually results in a lesser response of the nasal mucosa to external stimuli, presumably because all autonomic innervation is removed (Golding Wood, 1970). It would appear that



Fig. 6. Nasal mucosa of the dog after cervical sympathectomy

Table I *Changes in lower airway resistance following unilateral or bilateral superior cervical sympathectomy in the dog*

	Dog no.	Before operation (cm H_2O/l/sec)	After operation (cm H_2O/l/sec)
Unilateral vasomotor rhinitis			
Breathing through the mouth	1	1.60	1.90
	2	2.10	2.30
Breathing through the nose	1	4 10	5.00
	2	3.90	4.80
Bilateral vasomotor rhinitis			
Breathing through the mouth	3	1.24	1 92
	4	1.50	2.10
Breathing through the nose	3	3.54	4.95
	4	3 40	4.80

the vasomotor rhinitis produced in these dogs corresponds to the parasympathicomimetic reaction of the nasal mucosa. In this state the nasal mucosa reacts more strongly than normal to irritant stimuli. We used the more usual stimulus of cold air because patients with vasomotor rhinitis react strongly to cold. On other hand this parasympathetic reaction the nasal mucosa is produced also in allergic rhinitis, but is associated with other signs of allergy such as an increase in eosinophils in the nasal secretions and blood and this finding with the positive skin reaction, helps to differentiate allergic from nonallergic vasomotor reaction Sneezing was invariably obtained following cold air stimulation of the sympathectomized nostril more so than after similar treatment of the normal side This is most usual with vasomotor rhinitis and corresponds with the greater reactivity of nasal mucosa only under parasympathetic nervous influence However it must be remembered that the nose is the most important route for heat loss in the dog (Schmidt Nielsen et al., 1970) and this may have influenced these results. Vasomotor rhinitis has a distinct influence on the lower respiratory tract. While the number of dogs is not sufficient to make generalizations, pulmonary resistance was increased in all four dogs following induction of experimental vasomotor rhinitis, this was especially evident after a bilateral procedure. We think it is not only the obstruction of the nose which produces this influence. This conclusion is based on experience with patients suffering from vasomotor rhinitis. We tried to remove the obstruction by paralysing the nasal mucosa with 5% cocaine After this cocainization of the nasal mucosa the patients could breathe better through the nose but the nasopulmonary resistance still showed higher values Accordingly we think that besides the mechanical factor central reflex excitability is the most important factor in the relationship between the upper and lower respiratory tract. The parasympathicomimetic reaction of the nasal mucosa producing more reactivity of the nasal mucosa also increases the tonus of the tracheobronchial tree with subsequent increase in the pulmonary resistance For that reason vasomotor rhinitis has, potentially the possibility of producing changes in the lower respiratory tract which resemble bronchial asthma.

RESUME

La rhinite vasomotrice expérimentale uni- et bilatérale a été provoquée sur 4 chiens à l'aide de la sympathéctomie cervicale uni- et bilatérale Nous avons étudiés ce problème de plusieurs points de vue pour expliquer le mécanisme de la rhinite vasomotrice et la relation entre la voie respiratoire supérieure et inférieure.

ZUSAMMENFASSUNG

An 4 Hunden wurde durch eine einseitige oder doppelseitige Sympathectomie eine unilaterale oder bilaterale Rhinitis vasomotoria ausgelöst Der Verlauf und die Entstehung wurden studiert wobei besonders die Beziehungen zwischen den oberen und den unteren Atmungswegen untersucht wurden.

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DISCUSSION

A El Mofty We did similar work. I published 4 years ago the changes in the nasal mucous membrane after vidian nerve neurectomy showing disappearance of the oedema, regeneration of the cilia of the mucosa and increase in the number of the mast cells and the appearance of their intact granular contents 2 years ago we started a similar experiment cutting the parasympathetic supply of the nose of some dogs and eliciting changes like atrophic rhinitis. In other dogs we cut the sympathetic in the same way and, as expected, got changes like vasomotor rhinitis on one side and left the other side as a normal control. The mast cells are very few after sympathectomy and they were ruptured expelling their grounds. Their number was moderat in the normal control and was increased when the parasympathetic was cut. The parasympathetic was cut at the greater superficial-petrosal before it joins the general ganglion of the facial nerve We studied also the histopathological and histochemical changes. I would like to ask: did you find reduction of the nasal cells in your experiments or did you do any histochemical studies

U Fisch. Have you performed long-term experiments to find out how long the observed changes of the nasal mucosa will last? Our experience with the section of the major superficial petrosal nerve in vasomotor rhinitis indicates that the effect of this operation is limited in time.

T Palva In cervical sympathectomy the deep petrosal nerve that is supposed to carry the main sympathetic control to the nose is cut but a fair amount of the sympathetic fibres enter the nasal mucosa with the sphenopalatine and anterior and posterior ethmoidal arteries. One would think, therefore, that the effect of cervical sympathectomy would be short-lasting. In fact, some authors ascribe very little importance to the deep petrosal nerve: I wonder whether Mr Krajina did any stimulation experiments during surgery I should like to know whether unilateral ganglion resection produced a strictly unilateral vasomotor rhinitis. Cutting of the vidian nerve often causes changes also on the nonoperated side. Is there any experience of the effect of this procedure on ozena? If the product of swollen mucosa and increased secretion would be long-lasting, it would have a very beneficial effect upon atrophic rhinitis and ozena.

Z. Krajina (Reply) to Mr *El Mofty* Our intentions were not only to produce asomotor rhinitis by cervical sympathectomy but to study the relationship between upper and lower respiratory tract. Further more, we reported in 1961 (Padova) the results of cervical sympathectomy in rabbit. We could not find mast cells in the secretion of the nasal mucosa with this operation.

To Mr *Fisch* We followed the time of regeneration in human beings and animals This time is 3–6 months in human beings and in animals the symptoms abate after 4 weeks.

To Mr *Palva* We know that sympathicus reaches the nose via the cervical symp. plexus and through the arteries. That is the reason that apart from extirpation of the cervical sympathicus we removed periarterial symphat. branches around the carotid artery About the use of sympathectomy in atrophic rhinitis and ozena I have no clinical experience but I spoke many years ago with Professor Hamberger who used this method.

Acta Otolaryng 73 218–221 1972

SIGNIFICANCE OF ANTERIOR AND POSTERIOR TECHNIQUE IN RHINOMANOMETRY

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Abstract Posterior and anterior rhinomanometry are supplementary tests. Posterior rhinomanometry is less sensitive to disturbances caused by distortion of the movable external pyramid of the nose a common difficulty when testing caucasians. The significance of posterior rhinomanometry is greatly limited by the fact that it can be applied in only 50–60% of people. Very rare discrepancies in the information obtained by anterior and posterior rhinomanometry confirm the alue of the always applicable anterior rhinomanometry as a basic test. The frequent significant differences in the mean values of nasal pressure-drop or -rise by anterior and posterior technique never theless prove that these two different ways of measuring the nasal resistance are different tests and must be evaluated as such.

General acceptance and use of audiologic hods preceded the great progress of otology _ the two last decades. One reason for the slow development of rhinology during the same period seems to have been a lack of widely accepted nasal function tests. Most nasal respiratory tests have been designed for scientific purpose or individual special investigations (e g Drettner 1961 Ey 1970 Ogura & Harvey 1971 Stoksted, 1953) The demand to include a rhinomanometric test as an essen tial part of normal nasal examination was made by Cottle et al. (1963) Clinical applica tions have been developed by e g Akylldiz (1969) Guillerm (1967) Kortekangas (1971) Masing (1967) Montserrat Viladiu et al. (1967) and Spoor (1965). Nasal respirator function is highly variable because of the nasal cycle which has been recently reviewed by Keuning (1968). This variation in rhinomanometric tests makes a great difference in comparison with the audiologic tests with their very stable sensation levels.

In rhinologic respiratory function tests three parameters have usually been considered. Measuring the pressure fall during inspiration or the pressure rise during expiration effected by the nose is the basic test, the *pressure* test in rhinomanometry *Time* and the amount of air flow are the other usually measured parameters. The relation of the pressure change to the *volume* in time units can be expressed as one figure but estimating this nasal conductivity requires special expensive apparates (Spoor 1965). Two methods of measuring nasal pressures have been used, anterior and posterior rhinomanometry As discussions of the mutual value of these two methods are still going on this study was undertaken to serve clinical purposes.

MATERIAL AND METHOD

In posterior rhinomanometry a flow-free area is formed inside the posterior parts of the mouth. This area transmits the pressure variations unchanged through the pressure detector to the manometer (Fig 1). In anterior rhinomanometry the opposite nasal chamber serves a similar function (Fig 2)

There are different principles in anterior and posterior techniques. In anterior rhinomanometry the pressure difference between the nasopharynx and ambient pressure is recorded. In posterior rhinomanometry the pres-

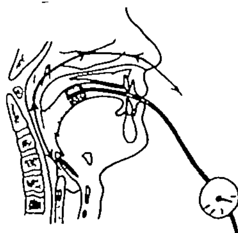


Fig. 1 A scheme of posterior rhinomanometry. The arrow depicts air-flow. The scattered area depicts a flow-free area.

sure difference between the mesopharynx and ambient pressure is recorded. This difference of the anatomical measuring points is, however insignificant, since this part of the "nasal respiratory tube" contributes very little to the pressure fall or rise which are mostly due to more distal parts of the "tube". A functional proof of this insignificance is given in Fig. 3. The lack of fluctuations when the pressure difference between anterior and posterior detectors is recorded proves that the difference between these two sites is insignificant.

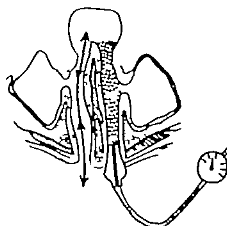


Fig. 2 A scheme of anterior rhinomanometry. The nasal chamber opposite the side under testing is a flow-free area.

A typical example of rhinomanometric recording is shown in Fig. 4. The following three parameters have been calculated from the curves:

(1) The inspiratory pressure fall: the mean height of the inspiratory culmination points from the baseline.

(2) The expiratory pressure rise: the mean height of the expiratory culmination points *from the baseline.*

(3) The sum of the pressures; the mean distance between the culmination points of successive inspirations and expirations.

The differences of the means were tested by Student's t-test and a level $0.01 > p$ was chosen.

Both normal-looking and pathological noses are included in this series. Selection was unavoidable as only those cases could be included in which the posterior technique could also be successfully recorded. Only results of two experienced examiners are included, the author being one.

In the treatment of the results only rhinomanometric findings have been considered. The pressures (i.e. deviations from the ambient pressure) less than 16 mm of water have been considered normal. The highest mean values encountered among the elevated pressures were 66.0 mm of water for inspiration and 43.8 mm of water for expiration. Each side of the nose is considered a separate examination, usually called a case. The assessment of a case in the normal or elevated group is decided by the mean inspiratory pressure.

RESULTS

Table I gives the general result of the comparisons of the anterior and posterior rhinomanometry in individual cases. The mean inspiratory pressure-drop differed significantly in 19% of the normal group and in 30% of the cases of the elevated group. The corresponding percentages for the expiratory pressure-rise were 15 for the normal and 33 for the elevated

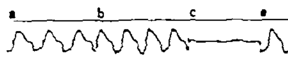


Fig 3 (*a*) posterior recording, (*b*) anterior recording, (*c*) pressure difference recording between naso- and mesopharynx, (*e*) anterior recording.

group For the sums of the pressures the percentages were 27 for the normal and 37 for the elevated group If the significance level had been determined at $0\ 05 > p$ the following percentages for differing mean values would have been obtained Inspiration. normal 27 elevated 42. Expiration. normal 25 elevated 59 Sum of the pressures: normal 38 elevated 52. As a properly performed rhinomanometric test is a precision test the more severe significance level, $0.01 > p$ is used throughout this udy

When the informative value of the result f the anterior and posterior technique is compared those cases which gave normal

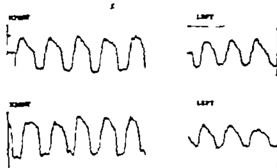


Fig 4 Actual recordings of anterior and posterior rhinomanometry The inspiratory phase is shown below the baseline and the expiratory phase above The calibration mark (in front of right anterior recording) = 20 mm of water

Table I *Distribution of cases according to the significance of the differences of the means of nasal pressure changes measured by anterior and posterior rhinomanometry*

Cases are grouped as "normal" and "elevated" according to the inspiratory mean value. If this is > 16 mm of water by either of the methods the case is classed as "elevated" *The significance is obtained by Student's t-test with the significance level* $0\ 01 > p$

	Insignificant difference	Significant differences: Anterior higher		Posterior higher		Total no. of cases
Inspiratory pressure						
Normal	39	4	8%	5	10	48
Elevated	32	10	22%	4	9%	46
Expiratory pressure						
Normal	41	5	10%	2	4	48
Elevated	31	10	22%	5	11%	46
Sum of inspiratory and expiratory pressures						
Normal	35	7	15%	6	13%	48
Elevated	29	12	26%	5	11%	46

pressure levels must be left out In any case the information was the same. Among the 10 cases in which the anterior technique gave a significantly higher inspiratory mean pressure-drop the posterior technique showed elevated pressure in 4 cases and normal pressure in 6 cases. In one of the latter cases there was an obvious discrepancy In the remaining 5 cases the pressure drop in the anterior test just exceeded the limit, the highest deviation being only 2.3 mm of water Thus in these 5 cases the information obtained by the anterior and by the posterior technique hardly differed. As upon close examination the results in all the other groups is about the same, it is not reported in detail. Altogether two cases were found in which the anterior technique gave information of elevated pressure while by posterior rhinomanometry normal or very slightly elevated pressure-drop was revealed. The apparent reason for both these discrepancies was distortions of the nasal valve region unavoidable in the anterior technique.

Table II *Incidence of significant differences between anterior and posterior rhinomanometry by the three parameters*

Significant difference in	Difference of the sums of inspiratory and expiratory pressures	
	significant	insignificant
Inspiratory pressures only		
Normal	7	—
Elevated	5	2
Expiratory pressures only		
Normal	3	2
Elevated	5	3
Both inspiratory and expiratory pressures		
Normal	2	—
Elevated	7	—

Only the difference of the sums was significant in one case

Table II shows the value of calculating the sums of the inspiratory pressure-drop and the expiratory pressure-rise In the only case in which the sum alone significantly differed, the difference in both inspiratory and expiratory values was close to significant ($0.02 > p > 0.01$) The reasons for insignificant differences for the sums when either inspiratory or expiratory values differ were often technical (distortion) but different mechanisms are involved in individual cases. Calculating the sum contributes very little to the evaluation. Both inspiratory and expiratory pressure changes are valuable as separate parameters and apparently are apt to reveal different pathological changes.

RÉSUMÉ

Quelques aspects de la rhinomanométrie du nez normal et du nez obstrué ont été étudiés. Une analyse statistique a été utilisée pour évaluer les résultats et pour comparer la valeur de la rhinomanométrie antérieure et postérieure comme indicateurs de pathologie nasale.

ZUSAMMENFASSUNG

Die posteriore und die anteriore Rhinomanometrie sind zwei Untersuchungen die einander ergänzen. Bei der posterioren Technik hat das Resultat weniger Neigung zum Einfluss der Distorsion der äusseren Nase, die sehr oft bei Kaukasiern schwer zu vermeiden ist. Die Bedeutung der posterioren Technik ist durch die Tatsache, dass diese Technik nur bei etwa 50–60 Prozent der Bevölkerung gebraucht werden kann, begrenzt. Da nur selten widersprechende Auskünfte bei anteriorer und posteriorer Technik gefunden wurden, ist die immer brauchbare anteriore Rhinomanometrie als eine wertvolle Grundmethode zu betrachten. Ziemlich häufige signifikante Unterschiede der Mittelwerte der inspiratorischen Druckverminderungen und expiratorischen Drucksteigerungen zeigen, dass die anteriore und die posteriore Rhinomanometrie doch zwei verschiedene Untersuchungen sind.

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Acta Otolaryng 73 222–226, 1972

FURTHER INVESTIGATIONS INTO THE IMMUNOLOGICAL ROLE OF TONSILS

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Abstract. The production of antibody by tonsils has been demonstrated previously by tissue culture technique after subcutaneous antigen administration. In the present study the immunological role of tonsils was investigated by two immuno-morphological methods. 1. The specific antibody producing cells were studied in tonsillar tissue after subcutaneous administration of antigen. 2. Antigen reception was studied after application of antigen to the epithelial layer of tonsils.

In our earlier experiments it was proved by tissue culture technique that the tonsils of hyperimmunized rabbits are able to produce ...fic antibody. Subsequently we have de...onstrated that human tonsils produce anti...dy and that they have immuno-memory. In these experiments the tonsils functioned as non-regional lymph nodes (Surján & Surján, 1969 *a, b,* 1971).

It is well known that the tonsils have no afferent lymph vessels. On this basis Falk (1963) categorically stated that the tonsils have no lymph-node function. According to our earlier investigation it was demonstrated that tonsils function as *non-regional* lymph nodes. But it has remained an open question whether the tonsils are able to work as *regional* lymph nodes, namely whether they are able to capture antigen or not. This latter problem was investigated by Goodale (1898), Kelemen & Hasko (1932) and many other authors. It was concluded by Naumann (1955) that there is no active resorption, but diffusion was observed in cases of crystalloids. The uptake of different bacteria was also investigated, but there are inconsistencies among the results of the different authors. These questions are reviewed in detail by Falk (1963).

From an anatomical and histological point of view the reticulated epithelium of crypts seems to be suitable for antigen reception.

The purpose of the recent study was to demonstrate whether or not antigen is able to penetrate into the tonsillar tissue.

MATERIAL AND METHODS

The above-mentioned problem was investigated by two different methods. Human gammaglobulin served as antigen and was applied to the surface of tonsils of rabbits through a submandibular incision. Thirty minutes later the tonsils were excised.

The first method was detection of this antigen by means of horseradish peroxidase labelled anti-human IgG.

The other method was the quantifying of the nuclear birefringence. This is a very sensitive indicator of the physiological condition of the cells.

Two groups of rabbits were investigated by these two methods. The first group was previously immunized with human gammaglobulin and 10 days later the two afore mentioned experiments were performed. In the other group of animals the antigen was applied to the ton-



Fig. 1 Lymphoid cells (*arrows*) situated in the reticular epithelium show peroxydaze enzyme activity on their surface.

tillar surface only without previous immuniza tion.

The tonsillar tissues were fixed in cold ethanol and embedded by a paraffin embedding technique of Sainte Marie (1962) for immunomorphological studies.

Antihuman IgG was labelled with horseradish peroxidaze according to Avrameas (1969).

Paraffin sections (8–10 μm) were treated with this labelled antisera, and peroxidaze enzyme-activity was demonstrated by the method of Graham & Karnovsky (1966)

Control sections were investigated without peroxidaze-labelled anti-IgG treatment.

Other sections were stained with neutral-red (0.1 M) and examined with polarized light. The quantity of the birefringence was measured with the method of Schmidt (1938). The path difference of birefringence structures was measured by means of a rotatory compensator (the path difference of which was 51 nm) ac cording to the $\Gamma = 51$ sin 2α equation, where α = the angle of compensation.

RESULTS

Observation with peroxidaze activity of previously non-immunized animals

(*a*) Rather strong peroxidaze activity was observed on the epithelial surface of tonsils and in a few cells in the lymphoid part.

(*b*) Moderate activity was found in the red blood cells and occasionally on the surface on the crypt-epithelium too.

(*c*) Weak activity was found in every cell of the sections, probably due to the non-spe cific adsorption of peroxidaze-labelled antiserum.

The strong and the moderate activities are produced by endogen peroxidaze enzymes be cause they were present in the control sections too It is therefore impossible to demonstrate the presence of antigen in the tonsils of non immunized animals.

Observations with immunized animals

In this group the same peroxidaze activities were seen as in the non-immunized animals, but, in addition, enzyme activity was detect able on the surface of some lymphoid cells situated in the reticular epithelium of the ton sillar crypts (Fig. 1) Such cells were never seen in the deeper part of tonsillar tissue.

We presume that the positively stained lymphoid cells have antibody molecules on their surface and that the antigen is captured by these antibodies.

The results demonstrate the penetration of antigen molecules into the epithelium of tonsillar crypts in cases of previous immunization.

However the immuno-histochemical method, using peroxidaze-labelled antibody was not successful for the detection of antigen in tonsils of non-immunized animals. This negative result may be caused by various factors:

This immuno-morphological method is not suitable—either essentially or in our own ex

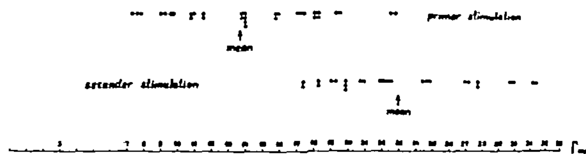


Fig. 2. Birefringence of tonsillar lymphoid cells after local antigen administration.

perience—for the detection of the micro-quantity of antigen which penetrated.

The penetrated globulin was possibly digested by phagocyte cells.

Investigations by means of polarized light

Birefringence of nuclei of tonsillar lymphoid cells was measured after local antigen administration in previously immunized and non-immunized rabbits. Results of 40 cells measured in both experimental groups are demonstrated in Fig. 2.

Birefringence is much greater in cases of ... nized animals.

The increase of nuclear birefringence is probably due to the alteration of the physico-chemical properties of DNP (Jobst & Kellermayer 1967). This phenomenon seems to us rather similar to the chromatin activation reaction. This term was introduced by authors from Sweden especially by Ringertz (1969). This reaction represents an early stage in the transformation of inactive chromatin segments into active templates for RNA synthesis.

Surprisingly enough both the chromatin activation reaction and the increased birefringence are detectable in the majority of lymphoid cells. But it is well known that after antigen administration only a minority of cells are able to take part in the specific RNA and protein synthesis. Therefore this early step of the nuclear activation is not a true specific reaction.

On the other hand it is important that this phenomenon is produced by the antigen but only in the immunized animals.

The increase of the nuclear birefringence in lymphoid cells means that after local administration the antigen penetrates the tonsillar tissue and produces the early signs of protein synthesis.

CONCLUSION

We suppose that our earlier and recent investigations help us better to understand the immunological role of tonsils (Fig. 3). We suggest that the tonsils take part in the immunological process as do the non-regional lymph nodes. This is demonstrated in the left part of this figure.

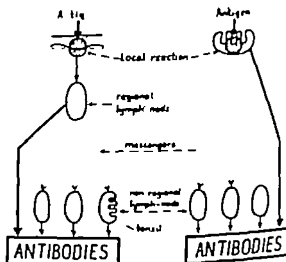


Fig. 3. Schematic representation of immunological functions of tonsils.

On the other hand the tonsils seem to be able to capture antigen from the oral cavity This too is illustrated, top right, Fig. 3 The purpose of the present study was to demonstrate the antigen uptake of tonsils. Our results seem to prove this. In our opinion, the tonsils are also able to function as regional lymph nodes. We consider that the tonsils, together with the gut-associated lymphoid organs and with the lymph nodes, play a very important part in immunity

RÉSUMÉ

Dans une étude précédente nous avons démontré dans une culture de tissu la production d'anticorps par les amygdales après injection sous-cutanée d'antigène. Dans l'étude présente le rôle immunologique des amygdales a été étudié au moyen de méthodes immuno-morphologiques. Les cellules produisant l'anticorps spécifique après injection sous-cutanée et l'absorption de l'antigène par l'épithélium de l'amygdale ont été étudiées.

ZUSAMMENFASSUNG

In unserer vorhergehenden Arbeit haben wir mit Hilfe von Gewebekulturen-Technik bestätigt, dass die Tonsillen nach Subcutan-Eingabe von Antigenen, Antikörper produzieren. In gegenwärtigen Untersuchungen haben wir die immunologische Rolle der Tonsille mit immunmorfologischen Methoden geforscht. 1 Wir haben die specifisch antikörperbildenden Zellen der Tonsille untersucht, nach subcutaner Eingabe von Antigenen. 2 Weiterhin haben wir die Aufnahmefähigkeit der Tonsille beobachtet so, dass wir die Antigene auf das Tonsillenephitel brachten.

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DISCUSSION

O. Sala. The problem is very interesting for our daily life. It is a common experience that some children are subject to recurrent infections of the Waldeyer ring and of the upper respiratory tract. Any surgical therapy is useless, if not dangerous. A pathophysiological presentation of the problem, as performed by Professor Surjan, is an absolute necessity. The behaviour of these structures is closely connected with that of the immunological system. I found alterations of the immunoglobulins (major and minor disimmunoglobulinias) in 68 out of 122 patients suffering from recurring infections of Waldeyer's ring. These children require systematic immunological study and intensive and extensive stimulation of the immunological defences.

L. Surján (Reply) to Mr *Sala*: I agree with you that tonsillectomy is contraindicated in cases in which the patients have immunological disturbances or the parents have a similar problem. The gut-associated lymphoid organs play an important role in the development of the immunological defence mechanism.

Acta Otolaryng 73 227–229 1972

THE CONTENT OF SOME TRACE ELEMENTS IN BLOOD SERUM OF PATIENTS WITH LARYNX CANCER

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Abstract The levels of four trace elements (iron, copper zinc, and cobalt) were investigated in 52 patients suffering from larynx cancer at different stages of the disease. The changes of iron content values in blood serum were additionally determined after oral load with iron preparation at 3 and 6 hours. The saturation coefficient as well as the latent capacity of iron binding were also determined. The results obtained prior to treatment and after laryngectomy are indicative of constant disturbances in iron metabolism in the first place and of considerable deviations from normal values for the other trace elements. The changes found seem to be one of the important factors influencing the morbidity from malignant tumours of epithelial origin in the upper air passages.

This report covers a part of a large number of investigations carried out in the Otolaryngological Clinic of the Medical Academy in Poznań on the metabolism of trace elements in diseases affecting mucous membranes of upper respiratory tract. The problem was the object of our report at the Collegium in Chicago 1967

Today I am going to concentrate on the iron levels in blood serum of patients with larynx cancer at different stages of its development. Although the role of outside factors in pathogenesis of cancer of the respiratory tract, especially of larynx and bronchi, is now beyond all doubt, efforts must be taken to come to know the local disposition of the mucous membrane to develop neoplasm, as this disposition in the process of carcinogenesis cannot be denied, either It is already well known that the metaplasia of the epithelia of many mucous membranes, preceding the development of cancer may result not only from outer irritations but may also be due to some inner factors. Iron deficiency even in subclinical forms of anaemia, causes a metaplastic change of the epithelium, which was proved on animals by the Hungarian hematologist Bernát (1965) the metaplasia is reversible on compensating the iron deficiency as proved experimentally by the same Bernát. Cancer of the larynx is a local disease for a fairly long time and thus seems to be a good model for making observations on the iron metabolism in the body as a possible antimetaplastic factor throughout all the stages of the disease.

We determined the iron level in blood serum also using the iron tolerance test and examining the latent iron binding capacity of the serum as well. The disturbances in iron level may result from poor supply of the element in food, its inadequate resorption in the alimentary tract as well as from some outer factors, among them also from substances contained in cigarette smoke, which has already been found. In the development of cancer of the respiratory tract, cigarette smoke might thus have not only its direct effect through the carcinogens contained therein, but also act indirectly interfering with humoral fluids and disturbing the iron metabolism in the body

Within the last 2 years we have examined 67 patients with larynx cancer (T_1 14 T_2 8 T_3, 35 T_4, 10). In T_1 group, 12 patients (86%),

Table I *Iron in blood serum of 67 patients with larynx cancer*

		Deficiency			Norm (males)
		Male	Female		
T	14	11	1	86	2
T	8	6		75	2
T	35	26	2	80	7
T	10	9	1	100	
Total	67	56		83	11

in T_2 group 6 patients (75%) in T_3 group 28 patients (80%), and in T_4 group 10 patients (100%) showed iron deficiency Out of the total of 67 patients in all the four groups the iron deficiency was found in 56 cases, i.e., in 83% To compare with the above the Hb level was found decreased in 52 patients, i e., in 78 there being a divergence in a part of patients between the two values. A decreased iron level frequently noticed in T_1 group of patients with rather limited local changes mostly without involvement of regional lymph nodes (N_0 – 12, N_1 – 2) speaks for a primary iron deficiency caused by endogenic or exogenic factors (smoking) rather than for the ry neoplastic disease In T_2 group comprising patients where only two of them showed ymph nodes involvement (N_1 – 2), the decrease in iron level found in 75% neoplasmic disease

As for T_3 and T_4 groups with considerable involvement of lymph nodes and advanced local cancerous process as well as with high *percentages of patients with iron deficiency* (T_3 – 80 T_4 – 100%) there is no foundation for finding primary iron deficiency as it can easily be seen in groups T_1 and T_2

Iron level determinations in patients operated on or irradiated, made several times in most cases, cannot be conclusive since the blood loss during extensive surgical intervention and the postirradiation reaction as well as blood transfusions following the surgical intervention considerably alter both the iron level in blood serum and the Hb level. It can generally be said that in cured cases the iron level did not undergo any significant changes when the cancer was eliminated.

Table II *Copper cobalt and zinc in blood serum of patients with larynx cancer*

		High deficiency	Moderate deficiency	Norm	Excess
Co	67	7	7	43	10
Co	36	6	14	16	
Zn	54	36	10	8	

In 67 patients the copper level, in 54 the zinc level and in 36 the cobalt level were determined and considerable derivations from the norm were found. The copper level in blood serum proved within the norm in 43 cases, was elevated in 10 cases, fairly lowered in 7 cases and considerably depressed in another 7 cases. The cobalt level was found normal in 16 cases; in 14 cases it was fairly lowered and in 6 cases considerably depressed. The most significant variations were observed in the behaviour of zinc. Its level in blood serum was found normal in only as few as 8 cases, being slightly lowered in 10 cases and considerably reduced in as many as 36 cases.

No interdependence could be pointed out as far as iron, copper and cobalt levels are concerned there was found, however some parallelism in the behaviour of iron and zinc levels.

Summing up the above findings it is to be stated that the patients with larynx cancer no matter how advanced the cancerous process in them did demonstrate considerable disturbances in the levels of trace elements in their blood sera, and especially in that of iron, which was not always depicted in the picture of clinical anaemia, and which may be of paramount importance for the local predisposing of mucous membrane in the form of metaplasia of the epithelium

RÉSUMÉ

On a examiné chez les malades avec cancer du larynx dans les différents stades de la maladie le niveau des 4 oligoéléments du fer du cuivre, du zinc et du cobalt. Ce que concerne le fer on a déterminé en outre le changement de son contenu dans le sérum

après une charge perorale a ec une préparation de fer après 3 et après 6 heures. On a aussi determiné le coefficient de saturation et la capacité de réserve pour la liaison du fer par le sérum. Les résultats obtenus indiquent clairement une présence des constants troubles avant tout du métabolisme de fer de même qu'une considérable déviation de l'état normal des autres oligoéléments. Les troubles constatés paraissent être un des très importants facteurs, qui influencent le développement des tumeurs malignes *d'origine épithéliale dans les voies respiratoires supérieures.*

ZUSAMMENFASSUNG

Man untersuchte bei den Kranken mit Kehlkopfkrebs in verschiedenen Krankheitsphasen das Verhalten des Niveaus von vier Spurenelementen: von Eisen, Kupfer, Zink und Kobalt. Was das Eisen anbelangt, so wurde ausserdem die Änderung seines Gehalts im Serum bestimmt nach peroraler Belastung mit einem Eisenpräparat, nach 3 und nach 6 Stunden. Es wurden ebenfalls der Sättigungskoeffizient und die Reserve eisenbindungskapazität durch das Serum bestimmt. Die erlangten Ergebnisse weisen deutlich auf eine ständige Störung vor allem des Eisenumsatzes hin, sowie auf eine erhebliche Abweichung von der Norm der übrigen Spurenelemente. Die festgestellten Veränderungen sind anscheinend ein unter anderen sehr wichtiger Faktor für die Beeinflussung der Entstehung von malignen Geschwülsten epithelialer Herkunft in den oberen Luftwegen.

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DISCUSSION

G. de Wit: Iron in the bloodserum is only on transport. The crucial question is, however, if there is an iron-deficiency in the tissue. One can detect a "hunger" of the tissue for iron by doing the "total iron loading test". The relation between the maximal loading with iron and the present content of iron in the serum indicates the hunger. Did Mr Zakrzewski perform such a loading test?

I. Friedmann: 1) Is the general level assessed in the normal population or in a control sample of normal persons? 2) Since heavy metals in higher concentration cause tumours of soft tissues would you comment on the significance of the low levels of cobalt observed?

S. K. Bosher: The serum copper findings seem a little unusual since in most of the neoplastic diseases so far studied, particularly the malignant lymphomas, the concentration is elevated. As the serum copper is present mainly as a structural component of ceruloplasmin, in α_2 globulin, I wonder whether he has any information about the protein metabolism in those patients with reduced serum copper concentrations.

A. Zakrzewski (Reply) to Mr *de Wit:* "The total iron loading test" has not been performed by us up to now. I am aware that the serum iron determination and all other parameters concerning the iron in serum show only the properties of transport iron, they are sufficient from the clinical point of view to state the iron deficiency of the body.

To Mr *Friedmann.* To the first question, we did not determine the norm for large groups of population. In small groups for instance, in a group of heavy smokers without a tumour but demonstrating the precancerous states in the larynx the deficiency of iron was very often stated. To the second question I am not able to comment on the low levels of cobalt.

To Mr *Bosher:* We have no explanation why the levels of copper were not so high as could be expected. Nevertheless only in some of our cases was the copper in serum reduced. In most of the cases the levels were normal or even elevated which is in accordance with the results of other authors who stated the deficiency of iron in these cases.

Acta Otolaryng 73 230–234 1972

ELECTROACOUSTICAL AND SPECTROGRAPHICAL ANALYSIS OF THE VOICE OF THE HUMAN NEO-LARYNX

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Abstract. The paper illustrates the results of a spectrographic research carried out on the voices of patients operated on with the technique of reconstructive laryngectomy and given a neo-larynx. The results show a great resemblance between the voice of the neo-larynx (composed of the first tracheal rings) and the physiological voice

The electroacoustical study of the voice of patients operated upon using the new technique of reconstructive laryngectomy allows the bringing to evidence of the voiced component which appears in the emission of these subjects, a voiced component caused by vibratory phenomena of the structures of the neo-
ix, excited, rather like a reed, by the flux
air
n the absence of these structures there would be only the possibility of a whispered voice with the known inconveniences of a very limited intensity the loss of every formal, and therefore expressive value and a limited intelligibility

On the other hand, the voice emitted by subjects laryngectomized according to the *usual techniques, or oesophageal voice, not* counting the use of prostheses, is composed of impulses with a very low frequency compared with the frequency of a normal voice which excites the supraglottic resonators periodically

The presence of voiced components, recorded by magnetic registration, must confirm the qualities which are noted when listening directly to the voice produced by patients operated upon using the reconstructive technique.

The problem appears vast and complex. different types of emission should be examined, choosing opportune phonemes or syllables so as to be able to build up a complete picture of the phonetic situation

In our study we limited ourselves to the examination of some vocal signals in order to draw some conclusions from them

On account of the nature of the operation, one may foresee that the voice emitted will be partly voiced and partly unvoiced an analogous situation to that observed in the case of some consonants emitted by a normal subject for example "V" "S" (sound), ʃ ("jour")

The first investigation may be carried out with the Sona-graph, which may already allow a sufficiently complete picture of the phoneme under examination The word we had chosen for our investigation was "AIUOLE" which presents the characteristic of being composed *of the 5 basic vowels of the Italian* alphabet. For an appropriate comparison, this word was pronounced by a normal subject in the two ways, normal laryngeal voice—voiced, and whispered vocie—unvoiced. In Figs. 1 (*a*) and (*b*) one may clearly observe the progress of the frequency components, which remains almost identical in the whispered voice, where there is no appearance—except some very slight traces—of voiced components.

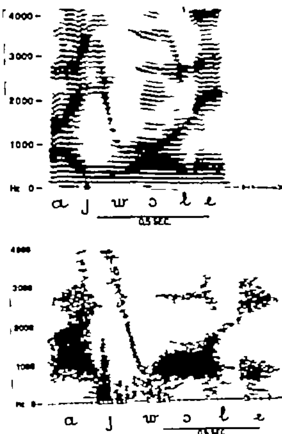


Fig. 1 (a) Normal subject. Spectrogram of the word "ajwale" emitted with a laryngeal voice (voiced). *(b)* Normal subject. Spectrogram of the word "ajwale" emitted with a whispered voice (Unvoiced).

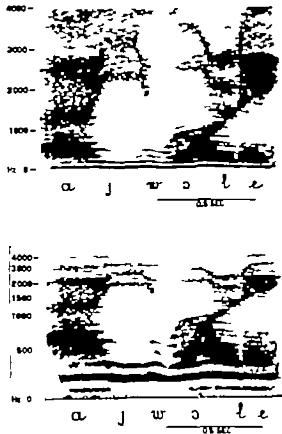


Fig. 2 Subject operated with reconstructive laryngectomy *(a)* Spectrogram of the word "ajwale" in linear scale. *(b)* Spectrogram of the word "ajwale" in logarithmic scale.

In a subject operated on with reconstructive laryngectomy (see Fig. 2 (*a*)) one can clearly see the appearance of the voiced component with the lower frequencies, and the unvoiced component with the higher frequencies, with a progress of components very similar to that of the normal subject. The voiced component goes as far as about 1 000 Hz, above which there is only an unvoiced situation.

In the diagram in Fig. 2 (*b*) in order to put the components with a lower frequency into better evidence, we have resorted to represent ing the frequencies in logarithmic scale, as this scale is a particularly wide one.

In another subject, in Figs. 3 (*a*) and (*b*), the two reliefs are repeated. whilst on one side there is an evident voiced component which covers a field of frequencies somewhat higher than the previous one, on the other side you may note a certain alteration of the rhythm of the word, perhaps due to insufficient educa tion of the subject and to respiratory difficulties which do not allow him to whisper all the components that permit a good intel ligibility of the word.

Visible-speech is useful for a global analysis of the phenomenon, but in some cases it may be opportune to resort to other techniques in order to make a better investigation of the nature of the phenomenon itself

The *oscillogram* shows us first and foremost the periodical nature of the phenomenon, revealing its fundamental rhythm, and at the same time it can give an idea of the possibility

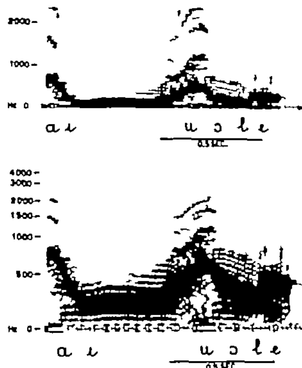


Fig 3 Another subject operated with reconstructive laryngectomy *(a)* Spectrogram of the word "ajwale" in linear scale. *(b)* Spectrogram of the word ajwale" in logarithmic scale.

...at there may be more or less distinct perio- ... phenomena in the zones of the formant ...

The oscillogram in Fig. 4 represents the prolonged vowel *a* in which the funda mental rhythm which turns out to be rather low about 60–65 Hz, is obvious.

In Fig. 5 the signal corresponding to Fig. 4 is filtered between 800 and 1 600 Hz, and in this way the periodicality of the component corresponding to the frequency of the for mant becomes much more evident

If an unvoiced component of the voice under examination were really present for every frequency the aspect of the oscillogram would be completely different, losing the character of regularity that it presents.

On account of the particular type of opera tion, one might wonder whether the formant structure frequency so obvious in the oscillo-grams, is actually a multiple of the frequency of the basic rhythm or not one could in fact

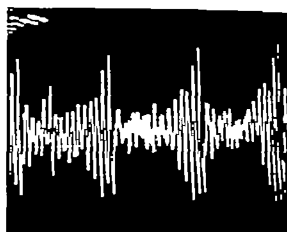


Fig 4 Oscillogram of the prolonged vowel *a* emitted by a patient subjected to reconstructive laryngectomy



Fig 5 The same oscillogram of the vowel *a* obtained with filtering.

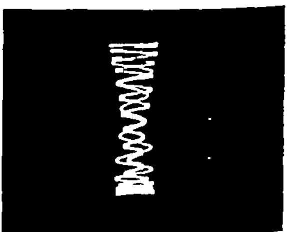


Fig 6. Vowel *a*—Lissajous figure.

observe a particular case, not possible in the normal larynx, but frequent in the sounds emitted by birds—that is the case of the contemporaneous emission of two periodic sounds that are not coherent with each other in other words, the anatomical structures of the neo-larynx could give rise to two sounds of diverse nature.

In one case so as to verify whether this phenomenon is also to be found in patients operated upon using the reconstructive technique, we put the signal of the fundamental tone, obtained by filtration, on the X-axis of the oscilloscope and the signal of formant structure frequency obtained through wide band filtration, on the Y-axis.

The fact that a perfectly stable Lissajous figure was thus obtained, as seen in Fig. 6 shows the coherence between the components with fundamental frequency and the components with formant structure frequency Summarizing we can say that the mechanism of the neo-larynx is largely analogous with the physiological mechanism of the normal larynx.

ACKNOWLEDGMENTS

The authors are grateful to Mrs C. Bordone Sacerdote, Ph.D and to prof. G. Sacerdote for their help in the interpretation of the experimental findings.

RESUME

La voix de malades opérés de laryngectomie reconstructive ayant un néo-larynx est décrite De ces recherches résultent des éléments de grande ressemblance entre la voix du néo-larynx (correspondant aux premiers anneaux trachéaux) et la voix physiologique

ZUSAMMENFASSUNG

Die Ergebnisse einer spektrographischen Untersuchung wurden beschrieben, die auf die Stimme von Patienten ausgeführt wurden, bei welchen eine rekonstruktive Laryngektomie mit einem Neo-Larynx ausgeführt worden ist. Aus den Analysen erben sich Elemente von grosser Ähnlichkeit zwischen der Stimme des neuen Kehlkopfes (der on den ersten Trachealringen dargestellt wird), und der physiologischen Stimme.

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DISCUSSION

J H Ogura Some years ago we published our tracheal shunt for the larynx in dogs. It works fine in the dog for airway voice and swallowing. We tried it in 3 humans but it was less successful. Swallowing was not so good and the operation was converted to the standard laryngectomy My understanding in your operation is that perhaps in 20% you are able to remove the tracheotomy tube. Would you tell me in how many of your 35 cases this has been permanent. Would you also tell me about the difficulties with deglutition, especially with solids and liquids.

J Tonndorf You have given us evidence that the neolaryngges are capable of producing voiced" i.e., periodic, sounds. However some of this evidence is on shaky ground. You showed oscillographic pictures, i.e., waveforms, of sounds, like the prolonged *a* Now when one looks at a waveform, one should not make statements about spectral components. The *a* had a pulsed waveform, and in such cases there may be wideband components present which one cannot tell from mere inspection. When filters are used the situation does not become much better Narrow filters pass quasi-period waveforms, and by inspection, once more, one cannot tell whether they are truly periodic. I believe you have proved your point, but the evidence was in your sonographic records, not in the presentation of waveforms.

J M Tato Il y a quelques années nous avons presenté au Collegium (il etait publié dans les Acta OL) le resultat de la recherche sur la spectrographie de la voix oesophagique chez les laryngectomies. Nous avons étudié quel était l'extension vocale, très limitée et qu avec un bon trainement est arrivée à avoir une extensive de 4 tones. Il est possible que l'extensive vocale soit meilleur dans le cas du neo-larynx Les auteurs ont-ils déterminé cette extension vocale? Ça peut-être un des éléments qui montre mieux les avantages parce que des fois ont trouvé très bons spectrogrammes dans les voix oesophagiennes.

M Arslan (Reply) to Mr *Ogura*: The latest check up held in July 1971 on the condition of the 35 cases of reconstructive laryngectomy we performed with the technique of Serafini, with the object of establishing exactly the present state of the three functions (phonation, respiration, deglutition), gave the following statistics. Obviously the parameters of judgement of these data are rather fluctuating on account of the following reasons: (1) with the passing of time, the functions generally tend to improve (2) there are both individual psychic factors and factors linked with the faithfulness shown by the patient in keeping scrupulously to the advice suggested to him for improvement; (3) the quality of the performance of the function (for example, one patient contents himself with moderate deglutition and is satisfied, another wants to be able to swallow all foods, even hurriedly and will never be satisfied, etc.).

Here are the percentages: 1) deglutition: 85% swallow well, even though at times slowly and in small mouthfuls; 2) respiration (inspiratory): 65% inhale normally (for safety about 40% of these are fitted with a very small cannula which is always closed); 35% still need to inhale through the cannula, but this number is tending to decrease; 30% have no cannula.

In the whole problem of reconstructive laryngectomy carried out with our technique one thing seems certain: that its indications do not exceed 20% in cases of laryngeal cancer We hope that other authors will know how to improve the technique so as to extend its indications.

To Mr *Tonndorf* I agree with Dr Tonndorf that the oscillogram I presented cannot give a definitive demonstration of the purely periodical qualities of the sound. The oscillographic research carried out by Dr Rossi had the sole aim of demonstrating that the vowel "a" of a subject operated with reconstructive laryngectomy is very similar to that of a normal voice.

To Mr *Tato*: It was not the intention of Dr Rossi's research to make a study of the vocal range of the subject operated with reconstructive laryngectomy since the fact, observed in our patients, that they can also sing, even though in a limited fashion, amply demonstrates that the vocal range is much larger than that of the laryngectomized patient who speaks with an oesophageal voice.

Acta Otolaryng 73 235–248, 1972

INNERVATION DENSITIES OF THE COCHLEA

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Abstract In normal cats the nerve fibres have been counted at different levels from the spiral ganglion to the outer hair cells by means of light and electron microscopy The relative numbers were calculated per millimeter cochlear length. Thus more exact information has been obtained about the ratio of the nerve supply to the inner and outer hair cells, the innervation density and the fibre branching in different cochlear turns. The great predominance of the innervation of the inner hair cells is confirmed. Only a small proportion of all nerve fibres leads to the outer hair cells. There is an overall innervation density maximum in the upper basal turn. About 95% of the afferent cochlear neurons are associated with the inner hair cells and only about 5% with the outer hair cells. The neurons associated with the outer hair cells have entirely different structural characteristics and do not undergo retrograde degeneration.

In earlier investigations we demonstrated in the cat that all upper tunnel radial fibres are efferent and that the entire afferent nerve supply of the outer hair cells is provided by the basilar fibres which cross the tunnel at the bottom. As a consequence of this innervation pattern it appeared that the great majority of all cochlear neurons are connected to the inner hair cells and only a small minority to the outer hair cells. These results were obtained from the evaluation of the afferent nerve fibres after elimination of the efferent nerve supply by section of the olivo cochlear fibres (Spoendlin, 1966, 1969) This surprising innervation ratio between outer and inner hair cells of about 1 20 could not be correlated to electrophysiological findings (Klang, 1965) and was therefore met with great reserve Since, however such an innervation pattern is of considerable importance for the functionin of the peripheral receptor we would like t present further evidence for it.

In order to avoid all possible errors whic might occur in degeneration experiments w proceeded to determine the total innervatio densities at different levels in the osseous spir lamina and the organ of Corti in the norma animal. To judge the ratio of innervatio densities between outer and inner hair cells w choose mainly two plans of section, one at th habenula perforata and the other through th area of the tunnel (Fig 1) In the first pla of section we find all fibres entering the organ of Corti and in the second only the fibres leading to the outer hair cells as upper tunnel radial fibres and basilar fibres (Fig. 2)

Difficulty in finding accurate reference points for distance represents the greatest problem to count reliably over greater distances these nerve fibres, which are only visible in the electron microscope. At the level of the outer or inner pillars these can be used as a reference scale and the numbers of the nerve fibres per pillar can be evaluated (Fig. 2 B) For 52 outer pillars we found an average of 3.5 tunnel crossing fibres per outer pillar Since there are about 3 000 outer pillars in one cochlea we can estimate the total number of tunnel crossing fibres to be about 10 500 fibres (Fig. 3)

The very irregular habenular openings can not, however be used as reliable reference points for distance. At this level we must there

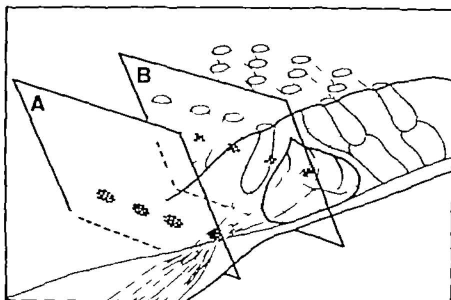


Fig 1 Plans of section used for the evaluation of the number of nerve fibres entering the organ of Corti (*A*) and crossing the tunnel (*B*).

fore use a different method for measuring large distances in electron microscopic sections. The mesh width of the grids used to support the ultrathin sections in the electron microscope is fairly regular with 84 ± 5 μ In low magnifications these grid holes can be depicted with the sections over them and we can count the number of nerve fibres per grid e (Fig 4). With this method, which is -pendent of other less reliable, reference uctures in the organ of Corti, we found in the basal turn 38 to 42 tunnel crossing nerve fibres per grid hole which is about 48 fibres per 100 μ cochlear length and 11 000 fibres over the total cochlear length of 23 mm in the cat. With both methods we get approximately the same figures for the tunnel crossing fibres.

The comparison of the number of tunnel crossing fibres with the fibres at the habenula per millimeter cochlear length reveals the following respective numbers of 500 to 3 500 in the basal turn and 300 against 2 300 in the second turn of the cat. In the guinea pig the difference is somewhat less pronounced with 550 against 1 750 fibres per millimeter cochlear length (Fig. 5) Thus we see that even in the normal animal without elimination of the efferent nerve supply we find, in the cat, only about one-seventh of all cochlear nerve fibres leading to the outer hair cells whereas the great majority of all fibres stay with the inner hair cells This confirms our earlier findings of a predominant innervation of the inner hair cells.

The restrictions of the electron microscopic examination are overcome by the use of light microscopic preparations, which allows us to evaluate the fibres over greater distances and not only in the organ of Corti but also in the bony part of the osseous spiral lamina. Sections can be made of plastic-embedded material

Fig .. (*A*) Low magnification electron micrograph of a plan of section corresponding to (*A*) in Fig. 1. The densely packed nerve fibre bundles (*N*) are clearly seen as they emerge from the habenular openings (*HA*) in the basilar membrane (*BM*). The only nuclei seen at this level belong to the supporting cells (*S*). (*B*) Low magnification electron micrograph corresponding to the plan of section (*B*) in Fig. 1 The upper portions of the outer pillars (*P*) are clearly seen. In the space of the tunnel run the fascicles of the upper tunnel radial fibres (*RF*) which are efferent, as shown earlier Hidden in the supporting elements above the basilar membrane (*BM*) are the afferent basilar fibres. The total number of all tunnel crossing fibres can be related to the number of outer pillars.

A

N

BM

B

P

RF

BM

outer pillars	upper tunnel radial fibres	basilar fibres	total number of tunnel crossing fibres
52	136	40	176

≅ 3.5 fibres per 1 outer pillar
→ [illegible] fibres per 3200 outer pillar

Fig 3 Numbers of tunnel crossing nerve fibres related to outer pillars.

without bone decalcification and in which it is easy to recognize every single myelinated nerve fibre These fibres are best counted with the aid of a counting chamber (Fig. 6) and it is easy to evaluate the number of nerve fibres in the osseous spiral lamina in different turns. It is clearly seen that the innervation density is highest in the middle and upper basal turn and lowest in the apical turn (Fig 7) The numbers of the tunnel crossing fibres varies less among the different cochlear turns, but they constitute in all turns only a relatively small proportion of all fibres entering the organ of Corti. The total number of nerve fibres at the habenula is only slightly reduced in significance after elimination of the efferent nerve supply Consequently we must conclude the number of efferent fibres is extremely small at this level and constitutes an almost negligible proportion of all nerve fibres (Spoendlin, 1970)

If we estimate the total number of afferent neurons in the cochlea on the basis of such countings we find an average of 2 000 per millimeter in the hook (~4 mm), 3 500 in the basal turn (~9 mm) 2 000 in the second (~6 mm) and 1 000 in the apical turn (~3 mm) which would give approximately 55 000 fibres for the entire cochlea, a figure which is slightly higher than those published by Gacek & Rasmussen (1957) and Schuknecht (1960).

The number of nerve fibres in the osseous spiral lamina remains more or less constant from the spiral ganglion to the habenula, which excludes considerable fibre branching along this course (Fig. 6)

In the basal turn the basilar fibres are about one-third of all tunnel crossing fibres whereas in the hook area and the second turn they are about half of all tunnel crossing fibres (Fig. 8). If we admit on the basis of our earlier investigations that the upper tunnel radial fibres are efferent, we can say that only about 5% of all afferent neurons provide the total afferent nerve supply of the outer hair cells. In earlier publications (Spoendlin, 1969 1970) we gave somewhat larger figures, which were based on a smaller amount of evaluated specimens and were related to the relatively smaller total number of afferent cochlear fibres according to Schuknecht (1960)

In view of this extremely small number of afferent neurons concerned with the innervation of the outer hair cells, the question arises whether these nerve fibres originate from special neurons different from the neurons associated with the inner hair cells.

A peculiarity in the degenerative behaviour

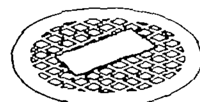


Fig 4 Schematic representation of a 200 mesh grid as used in the electron microscope with a section laying on it. The grid holes as such can be used as a reliable measure for distance

Number of nerve fibres per mm cochlear length

		fibres at habenula	tunnel crossing fibres
cat	first turn	~3500	~500
	second turn	~2300	~500
guinea pig first turn		1750	550

Fig 5 Comparison of the number of fibres at the habenula and the tunnel crossing fibres given per millimeter cochlear length.

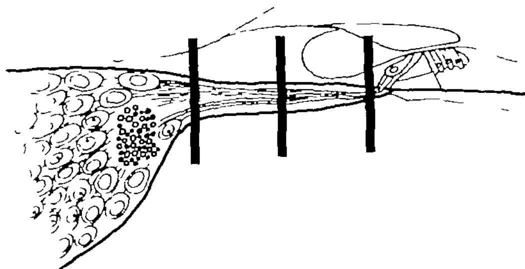


Fig. 6. Coordinate system superposed on a light microscopic pictures of a transverse section through the osseous spiral lamina in a plan of section as indicated in the schema. The myelinated nerve fibres can easily be recognized as small black rings and can be accurately counted with this method. The fibre density does not vary considerably at the different levels of the osseous spiral lamina taking into account the slight radial spreading of the fibres as they approach the organ of Corti.

of the cochlear nerve helps to clear this question. It has long been known (Ranson, 1905 Andres, 1961) that only about 50% of the bipolar *neurons in the spinal ganglion* undergo complete retrograde degeneration after transsection of the axons on either side of the ganglion. In the VIII cranial nerve we find a complete retrograde degeneration of almost all cochlear neurons, whereas only a small portion of the vestibular neurons de

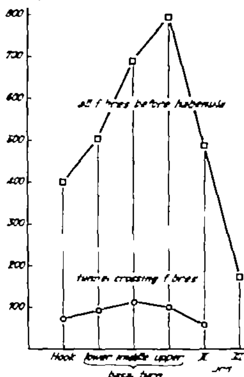


Fig 7 Graphic representation of the nerve fibre density at the habenula and in the tunnel for different positions in the cochlea.

..nerate after transsection of the VIII nerve ... the internal acoustic meatus Obviously ..ere are two different types of bipolar sensory neuron those that undergo complete retrograde degeneration and those that do not.

After transsection of the cochlear nerve in the inner meatus or after destruction of the peripheral axons only very few ganglion cells, scattered throughout the spiral ganglion of all turns, resist degeneration and survive (Spoendlin, 1971) (Fig. 9) These cells are however so few that they have been disregarded in earlier observations (Spoendlin & Gacek, 1963 1965)

Two types of bipolar ganglion cell have been described—in the acoustic ganglia of fish (Rosenbluth & Palay 1961) and in the spiral ganglion of guinea pigs (Kellerhals et al. 1967). In the cat at first sight all spiral ganglion cells seem to be alike and only after several investigations with serial sections was it possible to find two distinct types of ganglion cell (Fig. 10)

The great majority of all ganglion cells correspond to the large type I with a myelin sheath, a cytoplasm which contains besides mitochondria many ribosomes and much ergastoplasm and a large, regular round light nucleus with a very pronounced nucleolus. Only about 5% of all cells present an entirely different characteristic. These type II cells are small and difficult to find between the overwhelming majority of type I cells. They have no myelin sheaths, their cytoplasm contains many neurofilaments and the nucleus is often excentric, small, irregular frequently even lobulated and its nucleolus is less pronounced (Fig. 11)

Some 4 to 14 months after transsection of the cochlear nerve and degeneration of most spiral ganglion cells the few cells which remain appear in electron microscopic examination, to have all the structural characteristics of the

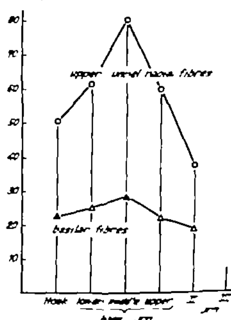


Fig. 8 Comparison of fibre densities of upper tunnel radial fibres and basilar fibres in the tunnel at different places along the cochlear turns.

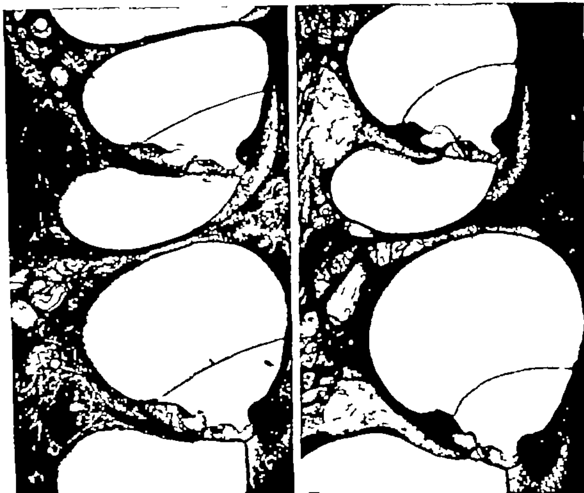


Fig. 9 (*Right*) Portion of a cat cochlea 6 months after transection of the cochlear nerve in the inner meatus. A large majority of all spiral ganglion cells have disappeared. However a few ganglion cells remain scattered throughout Rosenthal's canal in all turns. (*Left*), same portion of a normal cochlea with normal spiral ganglion cell-population.

unmyelinated small type II ganglion cells (Fig 12). Their total number corresponds again to about 5% of the entire normal ganglion cell population.

In the same animals we find the afferent innervation of the outer hair cells entirely intact, despite the complete degeneration of the 95% type I ganglion cells in the spiral ganglion. There is no appreciable reduction in the number of outer spiral fibres (about 100 per radial section) or afferent nerve endings on the outer hair cells, and these fibres and endings exhibit no signs of damage (Spoendlin, 1965 1971)

This combined survival of the 5% type II ganglion cells and the total afferent nerve supply to the outer hair cells obviously suggests that the peripheral afferent innervation of the outer hair cells and the ganglion cells of type II are related. In other words the entire afferent nerve supply of the outer hair cells derives from the 5% type II ganglion cells in the spiral ganglion (Fig. 15)

In fact we can follow in large interrupted serial sections the few remaining myelinated fibres through the osseous spiral lamina into the organ of Corti, where we find most of them to turn immediately after the habenula

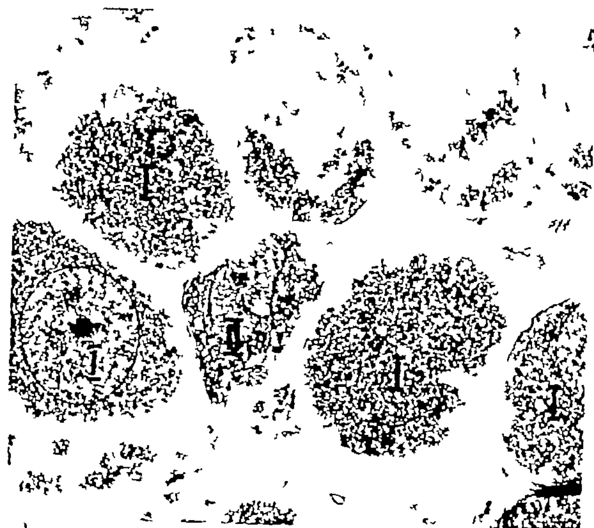


Fig. 10 Low magnification electron micrograph of a normal spiral ganglion of an adult cat showing a great number of type I ganglion cells (I) and a single type II ganglion cell (II) which is unmyelinated and small with an irregular nucleus.

basalwards for a distance of about 10 pillars before they penetrate between the inner pillars to cross the tunnel at the bottom as basilar fibres which finally form the outer spiral fibres with their afferent terminals on the outer hair cells (Figs. 13 A, 15)

There is, however also another type among the neurons which resist retrograde degeneration About every tenth habenular opening an especially large nerve fibre enters the organ of Corti proceeds radially towards the base of the inner hair cells and divides into two large branches running basalwards and apicalwards below the inner hair cell base making close contact with the sensory cells where we frequently find synaptic differentiations in form of synaptic bars. Their axoplasm contains a great many neurotubules and only a few neurofilaments in contrast to most other fibres in the organ of Corti (Spoendlin 1971). Each exceptionally large nerve fibre is connected to about ten neighbouring inner hair cells (Figs. 13 15) whereas each of the main inner hair cell neurons which derive from the 95% type I spiral ganglion cells is only connected to one inner hair cell. The total number of giant fibres to the inner hair cells corresponds to about 10% of all surviving neurons after

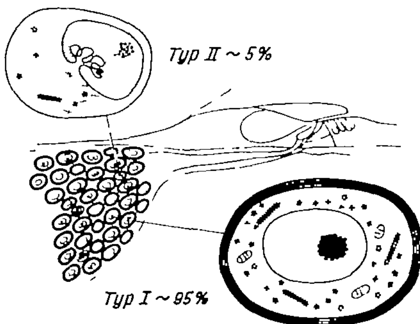


Fig. 11 Schematic re presentation of the two types of ganglion cells in the spiral ganglion and their relative percentage.

transsection of the cochlear nerve which is about 0.5% of all cochlear neurons.

CONCLUSIONS

All anatomical and structural evidence points to a numerically very predominant afferent innervation of the inner hair cells. About 95% of all afferent cochlear neurons are connected to the inner hair cells. Every inner hair cell is connected to approximately 20 afferent neu rons by means of unbranched radial fibres (Fig. 14) Every afferent neuron destined to the outer hair cells innervates about 10 outer hair cells by means of extensive terminal branching. The inner hair cells with their associated afferent nerve fibres represent a system with an enormous divergence whereas the system of the outer hair cells with associated nerve fibres shows a considerable convergence

The entirely different structural character istics and degeneration behaviour of the type I ganglion cells, related to the divergent inner hair cell innervation, and the type II related to the convergent outer hair cell innervation, might suggest that these two types of neurons also have an entirely different function. The enormous numerical predominance of the inner hair cell afferent innervation gives this system certainly the greatest quantitative importance. The convergent system of the outer hair cells, representing finally only 5% of all afferent cochlear neurons, could be an espe cially sensitive system as earlier suggested (Spoendlin, 1969 1971) However so far no electrophysiological evidence for such a small number of especially sensitive fibres has been found (Kiang, 1965)

Another possibility would attribute to the outer hair cell system mainly a control func tion over the inner hair cell system rather than a function of transmission of information to the central nervous system, as already theoret ically postulated by some electrophysiologists (Lynn & Sayers, 1970 Nieder & Nieder 1971) Intimate contact between afferent neurons from the outer hair cells and inner hair cells exists only immediately after or within the habenula, where these unmyelinated fibres are enveloped by a special type of satellite cell (Spoendlin, 1970). The influence which these



Fig. 12. A type II ganglion cell as found several months after transection of the cochlear nerve with degeneration of the great majority of all spiral ganglion cells (all type I cells). The typical outstanding characteristics of these type II ganglion cells are clearly seen. There is no myelin sheath around the ganglion cell but only a single Swann cell layer (*S*). The nucleus is excentric, irregular and even lobulated where it faces the main cytoplasm of the cell. The cytoplasm (*C*) contains, in contrast to the type I ganglion cell numerous neurofilaments. At the bottom, an offgoing axon (*A*) is seen.

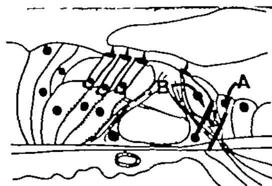


Fig. 13 (*A*) Longitudinal (tangential) section just after the habenula in the area below the inner hair cells 5 months after transection of the cochlear nerve with degeneration of all type I ganglion cells in the spiral ganglion. The very few remaining nerve fibres consist of some fibres (*N*) which run in a spiral fashion over a short distance and will continue as basilar fibres to the outer hair cells. In addition, at about every seventh habenular opening, a giant fibre (*GN*) is found. At the bottom the base of inner pillars is seen. (*B*) Longitudinal (tangential) section at the base of inner hair cells (*H*) shows one of the giant fibre branches (*GN*) contacting several hair cells and branching to some extent. Frequently the base of the inner hair cells presents irregular pr
around the branches of these giant fibres

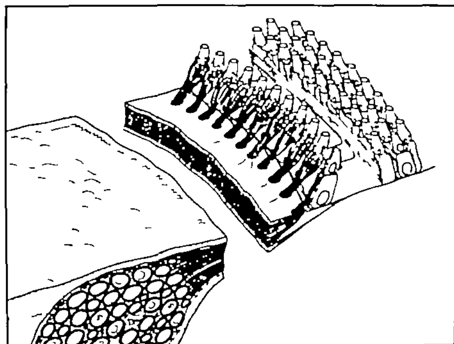


Fig 14 This schematic representation summarizes the afferent innervation pattern of the organ of Corti with the two types of neurons. 95% of all neurons are type I and are connected in a direct radial direction with the inner hair cells. Only 5% of all ganglion cells are of type II and constitute the afferent innervation system of the outer hair cells

fibres from the outer hair cells could have on the inner hair cell system would always originate from a group of outer hair cells located more basalwards on the basilar membrane This functional interpretation however remains an open question until further clarification of the interrelations of the different afferent fibres in the area below the inner hair cells and the habenula as well as the possible interference of the efferent fibres at this level is obtained. Studies related to these questions are under way in our laboratory by means of large reconstructions after selective elimination of the different innervation systems.

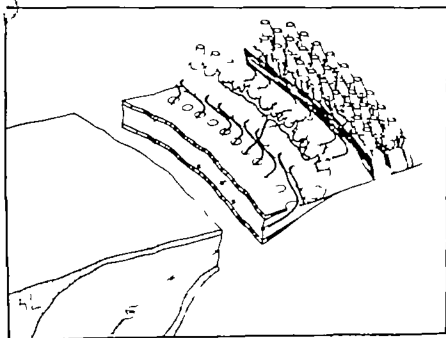


Fig 15 Schematic representation of the afferent innervation system of type II ganglion cells after elimination of the type I ganglion cells by section of the cochlear nerve in the inner meatus. The type II neurons provide exclusively the afferent nerve supply to the outer hair cells by means of the outer spiral fibres and their numerous collaterals to the base of the outer hair cells. In addition there are a few giant nerve fibres which connect several inner hair cells and which also belong to the type II neurons.

RÉSUMÉ

Dans les cochlées de chats normaux les fibres nerveuses ont été comptées à plusieurs niveaux différents à partir du ganglion spiral jusqu'à la région des cellules ciliées externes en utilisant le microscope ordinaire et électronique. Les nombres relatifs ont été calculés par millimètre de longueur cochléaire. On a pu obtenir ainsi des informations exactes sur la proportion des fibres nerveuses terminantes aux cellules ciliées internes et externes, sur la densité de l'innervation et sur les ramifications des fibres nerveuses dans les différents tours cochléaires. La grande prédominance de l'innervation des cellules ciliées internes est confirmée. Seulement une petite portion de toutes les fibres nerveuses de la cochlée arrive au niveau des cellules ciliées externes. Il y a un maximum de densité d'innervation globale dans la portion supérieure de la spire basale.

ZUSAMMENFASSUNG

Bei normalen Katzen wurden die Nervenfasern an verschiedenen Stellen vom Spiralganglion bis zu den äusseren Haarzellen mit Hilfe von Licht — und Elektronenmikroskopie gezählt. Die relativen Zahlen wurden pro Millimeter Länge des Ductus cochlearis ausgerechnet. Auf diese Art und Weise konnte man mehr exacte Information über das Verhältnis der Nervenversorgung der inneren und äusseren Haarzellen, die Innervationsdichte und die Verzweigungshäufigkeit der Nerven in verschiedenen Windungen der Cochlea erhalten. Die zahlenmässig weit überwiegende Innervation der inneren Haarzellen konnte bestätigt werden. Nur ein kleiner Teil aller Nervenfasern zieht zu den äusseren Haarzellen. In der oberen Basalwindung findet sich ein deutliches Maximum der allgemeinen Innervationsdichte.

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DISCUSSION

S. Iurato. I would like to suggest to Mr Spoendlin to try and follow the course of the fibres between the spiral ganglion cells he described and the outer hair cells on surface preparations, in order to have a more complete picture.

R. Thalmann. Until recently there has been hardly any physiological confirmation of Mr Spoendlin's provoking histological findings. I would like to draw attention to a paper by Ward & Duvall which was recently presented at the meeting of the Otological Society of America, and which I believe gives strong support to Mr Spoendlin's findings. Ward & Duvall exposed Chinchillas to octave band noise (1 000-

2 000 Hz) at 1.3 dB SPL for 15 minutes. This exposure produced a complete destruction of the outer hair cells in the first turn-and-a-half of the cochlea, but spared the inner hair cells. After the initial TTS had subsided, behavioral thresholds were completely normal. I would ask Mr Spoendlin to comment on these unexpected results.

M Portmann Remarquable travail qui apporte une contribution importante. Mais il n est pas necessaire d'imaginer une nouvelle théorie concernant la fonc tion des cellules ciliées externes. 5% seulement des fibres pour les cellules externes 95% pour les internes La théorie de H. Davis n est pas infirmée par ces constatations. En effet dans la courbe input output des réponses au clic, H Davis a imaginé que seules les cellules externes fonctionnent près du seuil et sont saturées vers 50 dB Il y a peu d'energie donc 5% des fibres suffisent pour la fonc tion de ces cellules. Après 50 dB la mise en jeu des cellules internes intervient, il s agit de fortes intensités donc de nombreuses fibres sont necessaires. Nos courbes ECoG chez l'homme atteint de recruitement s'inscrivent également dans cette théorie. Si l'on suppose que les malades avec recrutement ont une trouble exclusif des cellules externes, on trouve alors une réponse diphasique avec une deuxième phase tardive positive. Cette deuxième partie de la courbe (phase positive) pouvait ne pas apparaître chez l'homme normal car compensée par la réponse negative provenant des cellules externes simultanement en fonction. Donc je pense que le schema déduit des travaux de Spoendlin n'infirment pas l'ensemble de cette théorie, il peut au contraire encore s'y intégrer Il n est pas necessaire d'imaginer une autre fonction dite de controle pour les cellules externes à moins que des fait nouveaux tels celui que vient de citer Mr Thalmann ne viennent absolument infirmer les suppositions avancées par H. Davis.

J O Sala. Is it possible to explain the phenomenon of recruitment on the basis of your results

J Tonndorf My comment follows those of Mr Thalmann, on the Ward & Duvall paper and of Mr Portmann. We have gi en evidence in our laboratory for the fact that cochlear microphonics are the complex output of two separate groups of hair cells: group I (o.h.c.) more sensitive, linear input output function of lesser slope· group II (i.h.c.) less sensitive· linear input output function of greater slope Phase relationship between groups 120° < Δφ < 180° Evidence obtained mainly on the basis of the change of Δφ with amplitude (Similar evidence was obtained by P Dallos, Chicago who used *streptomycin* intoxication. Thus, the two groups of hair cells produce *different* kind of information, requiring a separate nerve supply)

H Spoendlin (Reply) to Mr *Iurato* We intend to follow these fibres not only in serial sections but also in surface preparations. A difficulty will be, however to stain these fibres reliably

To Mr *Thalmann*. I am very glad that more and more physiological findings can be correlated to the innervation pattern of the organ of Corti as demonstrated. Even in Bredberg's surface preparations of human cochlear there is often a much better correlation of the pure tone threshold curves of the audiogrammes with the state of the inner hair cells than of the outer hair cells.

To Mr *Portmann*. I fully agree that the concept of a very sensitive outer hair cell system and a less sensitive inner hair cell system is the best functional interpretation of this innervation pattern. I was brought to think also of other possibilities mainly because electrophysiologists like Kiang were never able to demonstrate a group of especially sensitive fibres in the cochlear nerve. In fact the curves of the electrocochleogramme with a flat portion at a middle intensity level and their typical changes in cases with recruitment where possibly the outer hair cell system is destroyed would be consistent with the concept of a minority of very sensitive fibres associated with the outer hair cells and a majority of less sensitive fibres associated with the inner hair cells. It must, however be kept in mind that there is not only a quantitative difference between afferent nerves to inner and outer hair cells but also a qualitative difference. It cannot be denied that the outer hair cell system could be an especially sensitive system and could have at the same time also a control function on the inner hair cell system.

To Mr *Sala*. There is a possible explanation of recruitment on the basis of a sensitive outer hair cell system and a less sensitive inner hair cell system with a different intensity range, as was earlier reported by us and discussed above by Portmann.

To Mr *Tonndorf* Although the cochlear microphonics cannot be directly related to the innervation pattern of the organ of Corti, the different input output curves for the CM show that even at the level of the sensory cells there is a different functional behaviour of outer hair cells and inner hair cells.

Acta Otolaryng 73 249–253 1972

PATHOGENESIS OF INNER EAR LESIONS IN ACUTE ACOUSTIC TRAUMA

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Abstract Clinical and morphological experimental data are presented concerning the development and final extent of inner-ear damage after acute noise exposure. Possible means of influencing the course of hair-cell degeneration and the theoretical and practical relevance of the findings are discussed. The results suggest modifications of the accepted concept of the pathogenesis of acute acoustic trauma.

Whenever a patient consults you for help after an acute acoustic trauma, you have to confess your inability you may tell him that his future hearing will be subject to an unpredictable fate By chance the patient's initial hearing loss will signify mainly a mere temporary threshold shift, or it may remain as a permanent hearing defect. You may conclude the consultation by informing your patient that no treatment will change the course of this development.

Theoretically however there *must* exist a possibility to diminish the extent of permanent hearing loss after an acute acoustic trauma, although nobody can expect to find a treatment to resuscitate irreversibly damaged cochlear hair cells. After exposure to damaging noise intensities, the cochlear hair cells commence a relatively slow degeneration process, and many of them cross the limits of irreversible degeneration stages after several days only The early still reversible degeneration stages present a considerable lapse of time that appears to be suited to therapeutic attempts at saving the degenerating cells before they are lost for ever

Experimentally we can show that in noise-damaged guinea pigs, the number of destroyed hair cells in 45 cochleogram counts presents a well marked statistical tendency to increase over many days (Fig 1). All results are affected by an enormous variance, due to the well known fact that both the individual susceptibility to noise damage and slight variations of the anatomical features imply an uncontrollable variability of the resulting amount of destroyed hair cells. Comparing the evolution of the functional damage and the morphological hair cell losses, we have to stress an unexplained discrepancy the functional damage is worst immediately after the damaging noise exposure whereas (except for some known reversible changes like the swelling of dendrites below the inner hair cells) the morphological damage slowly increases. After many days only functional and morphological damage become congruent.

All these considerations imply the conclusion that the search for an effective treatment of acute acoustic trauma has to be continued. In analogy to the favourable results of low molecular weight dextran in cases of vascular sudden deafness, we started to try the same treatment in acute acoustic trauma (Hippert & Pfaltz, 1970 Kellerhals et al., 1971) Our clinical and experimental trials resulted in the following findings (Table I).

Experimentally guinea pigs were exposed to a series of gun shots. Five days later the total number of destroyed hair cells was counted, using the cochleogram method according to

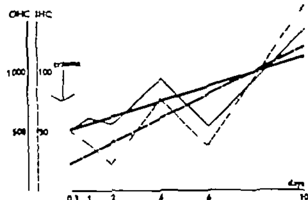


Fig. 1 Increase of the number of collapsed hair cells after acute acoustic trauma (gun shots). — outer hair cells, inner hair cells the thin lines connect the means of several cochleogram counts thick lines represent the overall regression lines.

Table I *Mean numbers of destroyed hair cells per cochlea*

	Outer hair cells	Inner hair cells
17 untreated animals	1 43	116
12 dextran-treated animals	478	26
8 albumin-treated animals	630	45

Untreated > dextran, $p < 0.01$ (OHC), $p < 0.05$ (IHC)
Untreated > albumin, $p < 0.10$ (OHC).
Dextran < albumin, not significant.

Engström (Engström et al. 1966) Untreated animals showed severe hair cell losses, whereas animals pretreated with intravenous infusions of low molecular weight dextran suffered much smaller numerical hair cell losses. The difference between both groups is statistically significant for both the inner and outer hair cells. The same favourable results were obtained after the administration of an albumin solution with the same water-binding capacity

As an example of our clinical results we may present the audiograms of a group observation of inner ear damage after an explosion in a major Basel chemical plant (Fig. 2) The untreated group showed a statistically significant lower gain than the group treated with a series of dextran infusions ($p < 0.05$)

Clinically and experimentally low molecular weight dextran diminishes the extent of permanent hearing loss after an acute acoustic trauma Besides its practical value this fact appears to imply a new concept of the pathogenesis of inner ear lesions after acute exposure to damaging noise intensities. Low molecular weight dextran is known to improve the capillary blood flow in several ways. in normo-volemic patients, its water-binding capacity raises the plasma volume far beyond the normal state the cardiac output increases, raising the flow of blood into the capillaries. At the same time, low molecular weight dextran diminishes the blood viscosity by hemodilution the hematocrit value falls by 40% and aggregation of erythrocytes or other blood corpuscles is less likely to develop. Albumin exerts the same effects, whereas the not generally accepted specific anti-sludge effect concerns dextran solutions only

For capillary hemodynamics, viscosity changes are of vital importance: for the circulation in the larger vessels, viscosity changes can be neglected, and blood may be regarded a Newtonian fluid. But in the capillaries, viscosity may multiply as much as 40 times in low flow states, giving rise to a vicious circle enabling the red blood cells to aggregate

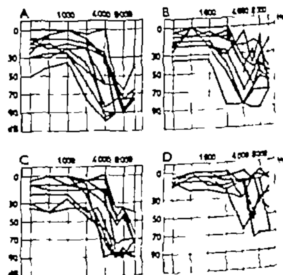


Fig. 2 Audiograms (bone conduction threshold curves) of the control group (*A*) and the dextran-treated group (*B*) immediately after the explosion and 1 month later (*C*) control group, (*D*) dextran-treated group



Fig. 3 Unstained surface preparations of the stria vascularis (*a*) strial capillary in normal animal, (*b*) strial capillary 4 hours after acute acoustic trauma. *L* = leucocyte.

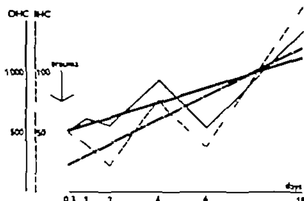


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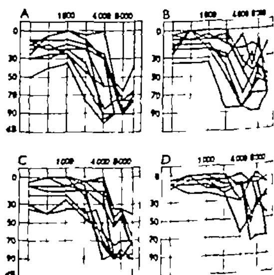


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DISCUSSION

M Lawrence: A temporary threshold shift, no doubt, can be the result of alteration in the microcirculation, and sound may be one of the factors that can produce these vascular changes. There are however many variables that could produce different results in different experiments. In our experiment reported a couple of years ago we used pure tone and monitored the sensitivity shift in the animal ear by recording the a.c. potential. When a 15 dB shift was produced the animal was sacrificed and the microcirculation examined. We found capillaries empty as the result of swollen endothelial cells in both the stria vascularis and the basilar membrane. Mr Kellerhals used gun shots to produce the trauma and such an impact sound may have a different effect than a pure tone. Another factor that could affect the results is the time and method of fixation. I have watched the microcirculation as the animal dies and found that the capillaries increase their diameter and become packed with red cells. It would appear that in order to get a true picture of the microcirculation the ear should be fixed practically while the animal is still alive. Would you elaborate a little on your method of fixation used in your animals?

A Appaix: Je voudrais demander si vous n avez pas essayé de traiter ces cas par oxygénothérapie hyperbare, qui s avère un excellent moyen d'amener de l'oxygène en excès aux cellules. Il y en a deux preuves cliniques la guérison constante des gangrènes gazeuses, par mort des microbes anaérobies; et plus près de notre domaine, les résultats dans les surdités brusques non traumatiques, qui si le traitement est précoce, sont très satisfaisants, et très probablement par l'apport d'oxygène à des cellules en difficulté.

O Sala: Patients who have suffered an acute acoustic trauma are usually considered as lost patients. I treat these patients as early as possible by medical therapy: heparine, ysotol at high doses, nicotinic acid, antiserotonin drugs, oxygen carriers, for 20–30 days. This therapy may influence vascular disturbances, extracellular oedema, biochemical changes of the cells less damaged by the acute acoustic trauma. Some improvement of hearing function can be obtained.

H Spoendlin: I would like to stress the point, raised by Lawrence, of the way how the animals were sacrificed and fixed. Even by simple decapitation the degree of filling of the inner ear vessels can vary greatly according to the state of excitation, anaesthesia and position in which the animal has been sacrificed. It might well be that microcirculatory disturbances are the cause of early acoustic trauma threshold shift but only below the critical intensity for irreversible mechanical damage, which we showed to be about 125 dB for white noise.

F Escher: May we still admit today a protective action of Vitamin A as was discussed 15 years ago?

D Hood: Susceptibility to noise-induced hearing loss varies very considerably from one subject to another. At Queen Square we have adduced evidence that susceptibility may be related to the hearing level itself. In other words the more acute the hearing the less the susceptibility: the less acute the greater the susceptibility. As I understand it dextran not only aids recovery from noise-induced hearing loss; it also acts as a prophylactic measure in lowering susceptibility. If this is so, then it follows that dextran, by improving the microcirculation, should increase the hearing sensitivity and I should like to ask Mr Kellerhals if he has any evidence of this.

B Kellerhals (Reply) to Mr *Lawrence* and Mr *Spoendlin*. Problems of fixation artefacts and filling of the strial capillaries in agony have been carefully taken into account, all animals have been sacrificed and processed the same way. The difference in morphological appearance was thus considered to correspond to real *in vivo* differences.

To Mr *Appaix*: I know your paper includes one case of acoustic trauma, but I think the effect of low molecular weight dextran (34% increase in cerebral blood flow according to Gottstein) exceeds the favourable effect of high pressure oxygen therapy.

To Mr *Sala*: The same considerations apply to the polypragmatic therapy as was proposed by Dr Sala.

To Mr *Escher*: We have no clinical or experimental experience concerning the protective effect of Vitamin A. According to literature, Vitamin A may improve the hearing loss due to Vitamin A deficiency but excess of Vitamin A probably exerts no significant sound protective effect.

To Mr *Hood*: We have not studied the effect of dextran upon the audiometric curves of normal-hearing persons.

Acta Otolaryng 73 254–258 1972

MACROSCOPICAL AND MICROSCOPICAL CHANGES IN THE BOTTOM OF THE INTERNAL AUDITORY MEATUS

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Abstract. Our macroscopical findings in the region of the deeper part of the internal auditory meatus in presbyacusis have been proved also on microscopical sections. The apposition of bone substance with advancing age, causing the compression and atrophy of the acoustic nerve fibres and the reduction of the ganglionic cells has been found also microscopically in cases of clinical presbyacusis.

In 1958 we published (Sercer & Krmpotić) a new concept of presbyacusis based on examination of one part (750 specimens i.e. 1 500 temporal bones) of the large osteological collec of Perović ranging from the fetal life to years of age

We have found that with aging in the fundus of the internal auditory meatus a progressive bone apposition occurs resulting in reduction of the number of holes for the nerve fibres in the spiral tract, and very rarely also in the vestibular area. These changes occurred first in the region of the basal coil and this fact was linked with hearing loss for high tones in advanced age The osseous cuffs surrounding the arteries in the region of the spiral tract in its basal and occasionally modiolar part were considered as a further proof of th apposition of bone progressing with age In very rare cases the bone apposition went so far that it closed completely the nerve openings of the spiral tract, resulting, no doubt, in complete hearing loss. On sections through the nerve closest to the spiral tract where the nerve follows exactly the contours of the spiral tract we demonstrated that the reduction of holes in the tract matches the reduction of the nerve fibres in the corresponding part of the nerve.

We studied additionally the remaining 1 100 macerated temporal bones of the above mentioned collection of Perović in Zagreb. We thus examined altogether 200 fetal temporal bones, 400 temporal bones from newborns to 25-year-olds and 2 000 temporal bones from persons aged 26 to 90 years. In all specimens the bottom of the internal auditory meatus was exposed by enlarging the channel by drill (Krmpotić, 1971)

At the University of Chicago in the Audiological Foundation in Los Angeles we had opportunity to check my macroscopical findings on the temporal bone collection of the Otological Laboratory We have examined serial sections of 174 temporal bones from 87 individuals in the age range from newborn to 90 years. The specimens were decalcified and mounted in celloidin, and stained with hematoxylin-eosin Most of the sections were horizontal very few were vertical

For more than one-half of the investigated specimens audiograms were available and for the rest a short case history so that the findings on the slides could be correlated with the recordings in the audiogram or the case history Cases with otosclerosis or any other disease of the ear other than presbyacusis and



Fig. 1 Ganglionic groups in a 65-year-old man (H-E, 40).

normal ears, were not taken into consideration.

The findings on these serial sections corresponded completely to the macroscopical observations on the osteological collection.

In young individuals the channels of the spiral tract are wide, and the nerve bundles large and in contact with walls of the channels. There is only a slight apposition of osteoid in the fundus between the openings of the nerves.

The cells of the spiral ganglion form a compact ganglionic mass. By slight but constant apposition of the dense fibrous tissue, osteoid and bone substance in the fundus, the region of the spiral tract belonging to the basal coil of the cochlea thickens. Consequently the nerve bundles passing through the holes of the spiral tract are separated and the peripheral nerve fibres compressed. This separation of the nerve bundles is followed by atrophy and disappearance of the peripheral nerve fibres, resulting in a secondary atrophy of the peripheral ganglionic cells. The ganglionic cells thus form several groups distant from each other with each group corresponding to a bundle of nerve fibres (Fig. 1). From 10 to 20 years of age this grouping of the ganglionic cells can already be very clearly seen. Cajal already expressed the opinion and demonstrated that fibres of equal size, lying next to one another could have different degrees of vulnerability. He showed in experiment on sciatic nerve that the peripheral fibres were, in ligature, more severely damaged than the central axons which seemed to be spared.

In older individuals, osteoid and bone apposition, respectively are localized at the entrances of the nerve channels. The new bone compresses the peripheral fibres of a nerve bundle. At the same time the apposition of bone makes the surface of the fundus uneven and rough. After the 50th year the space between the singular nerve bundles and the bony wall of the channels is always occupied by osteoid tissue or bone entering the channels (Figs. 2, 3). The circular compression of the nerve bundles can be seen distinctly. The fibres on the periphery of the bundles become atrophic while the more centrally lying fibres are still well preserved. The progressive atrophy of



Fig 2 Compression of nerve bundles by osteoid in a 59-year-old man with presbyacusis (H-E, 40).

the nerve fibres results in a secondary progressive atrophy of the ganglionic cells on the periphery of the ganglionic groups.

In advanced age (between 80 and 90 years) the channels for the nerve bundles become al- closed by osteoid and contain only a few no nerve fibres at all. Bone apposition around the arteries in the fundus can also be seen (Fig 4). Bony cuffs surrounding the arteries seem to compress them The bony cuffs which we have previously described on macerated bones as empty cylinders protruding into the internal auditory meatus are present especially in the region of the basal coil.

In very old persons the osteoid apposition can be seen occasionally also in the saccular area compressing the peripheral bundles of the vestibular nerve.

In the case of atrophy of the cochlear nerve for toxic or traumatic reasons in young as well as in elderly individuals, no apposition of bone occurs in the almost empty nerve channels. The empty space in the channels is filled with loose connective tissue entirely different from that in presbyacusis.



Fig 3 Complete closure of some openings for nerve bundles (left) in the spiral tract of a 76-year-old man with presbyacusis (H E, 35).



Fig. 4. Bony cuffs around arteries in fundus of a 82 year-old man with presbyacusis (H-E, ×40).

This bone apposition and atrophy of the nerve fibres and ganglionic cells is part of a general biological process in advanced age in the region of lamina cribrosa, openings for the second and third branch of the trigeminus, canalis facialis, foramina intervertebralia, etc. The compression of the arteries in the fundus by newly-formed bone causes disturbances in the vascularization resulting in atrophy of the stria vascularis and organ of Corti. This concept of presbyacusis could thus explain all four types of senile impairment of hearing.

It is interesting that Gussen (1969) has recently found also plugging of the vascular canals in the otic capsule due to the aging process.

RÉSUMÉ

Nous avons pu confirmer nos investigations macroscopiques du fond du conduit auditif interne en presbyacousie par des séries de coupes microscopiques. L'apposition osseuse dans la vieillesse, qui cause d'abord une compression et ensuite une atrophie des fibres nerveuses de nerf auditif avec une réduction du nombre des cellules ganglionnaires, a été retrouvée microscopiquement en cas de presbyacousie clinique.

ZUSAMMENFASSUNG

Wir konnten unsere makroskopischen Befunde am Boden des inneren Gehörganges bei der Altersschwerhörigkeit auch an mikroskopischen Schnittserien bestätigen. Die Knochenablagerungen während des Alterprozesses, welche die Kompression der akustischen Nervenfasern, deren Atrophie und Schwund der Ganglienzellen verursachen, wurden auch mikroskopisch in den Fällen, wo klinisch Presbyacusis festgestellt war gefunden.

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DISCUSSION

R. Hinchcliffe: Are we to understand that the mechanism of bony compression is the principal func-

tion in the etiological presbyacusis. And secondly as you know a deterioration in the thresholds of sensation is not specific for audition but characterizes other sensory modalities also. Do the authors consider that this same similar mechanism of bony compression underlies the determination of other sensory thresholds

M van Eyck. Mme Krmpotić a-t-elle également observé un rétrécissement du trou optique. Car il y a une grosse différence entre la presbyacusis et la presbyopie. Dans la presbyopie il s'agit d'altérations du cristallin sans forts altérations du nerf optique lui-même puisque la presbyopie est parfaitement corrigée par le port de verres.

C R. Pfaltz: Some years ago we examined the vestibular function in elderly people. Although the audiogram always showed the typical symptoms of presbyacusis the vestibular threshold as well as supraliminar responses were always normal. Were there any anatomical differences with respect to some opposition and narrowing of the bony canals at the side of the vestibular and cochlear portion of the VIII nerve which might explain our observations

B H Colman. Were you able to examine material obtained from old people who were known to have had normal hearing? If so, what were your findings in the bone of these individuals?

J Krmpotić (Reply) to Mr *Hinchcliffe.* I believe that this bone apposition can be considered as the cause of presbyacusis. The bone apposition is found also in other regions where the sensory nerves or nerve fibres pass through bony openings or channels as in lamina cribrosa (causing presbyosmie) or vestibular area (chronic presbyostasis).

To Mr *van Eyck.* We are studying now in our department also the other nerve and vessel openings in the region of the base of the skull f.i. can. opticus, foramen spinae foramen mentale, etc and it seems that there occurs also a progressive narrowing of the openings due to advanced age

To Mr *Pfaltz.* We have also found bone apposition in the vestibular area but it does not occur as often as in the region of the spiral tract.

To Mr *Colman.* We have also found very old people without bone apposition in the region of the spiral tract and who had good hearing, according to their case history

Acta Otolaryng 73 259-266, 1972

DEGENERATIVE CHANGES OF THE ARTERIAL VESSELS OF THE INTERNAL AUDITORY MEATUS DURING THE PROCESS OF AGING

A Histological Study

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Abstract Degenerative changes are observed histologically in the wall of the arterial vessels of the internal auditory meatus following the first decade of life in man. These changes consist mainly in a progressive thickness of the tunica adventitia accompanied by an increase of collagenous tissue and loss of fibroblasts. The increasing compactness of the adventitial layer may lead to complete acellularity and loss of structure (hyalinization). A possible functional implication of the adventitial changes observed in the arteries of the internal auditory meatus of the aging men may be a difficulty in contractibility related to the increasing amount of collagenous or hyaline tissue present in their walls.

The histological changes occurring during the aging process in the arterial system of the internal auditory canal have not yet been specifically investigated. Bosatra (1956) described minimal quantitative and qualitative modifications (particularly subendothelial hyperplasia of the tunica intima and hypertrophy of the internal elastic membrane) in the wall of the "internal auditory artery" of the aging man. The vascular segment investigated by this author was, however removed outside of the internal auditory meatus and according to its position, ramification and size corresponds to a branch of the anterior inferior cerebellar artery only partly involved in the blood supply of the internal meatus itself[1] The purpose of this investigation was to describe the histological structure of the arterial vessels of the internal auditory meatus during the different decades of life Particular attention was paid to those vessels carrying the blood to the inner ear

[1] In order to avoid confusion, the official anatomical nomenclature has discarded the name of "internal auditory artery" as early as 1955 (Fisch, 1969).

MATERIAL AND METHOD

78 temporal bones without apparent pathology other than the changes imputable to the age of the patients, were selected from temporal bones collection of the E.N.T Department of the University of Zurich. The age of the patients ranged from 2 weeks to 88 years. The distribution of the specimens according to the decades of life is shown in Table I. On each specimen representative sections of three categories of small arteries *lying* in the internal auditory meatus were taken (Fig. 1)

(*a*) Vessels with an average outer diameter of 150 microns, corresponding to the labyrinthine artery or arteries.

(*b*) Vessels with an average outer diameter of 100 microns, corresponding to the arteria cochleae propria or arteria vestibuli anterior

(*c*) Vessels with an average outer diameter of 60 microns, corresponding to the Vasa nervorum suppling the VIII nerve

The diameter of the vessels as well as the thickness of their vascular layers were measured using a Zeiss screw micrometer ocular The investigated sections were stained with

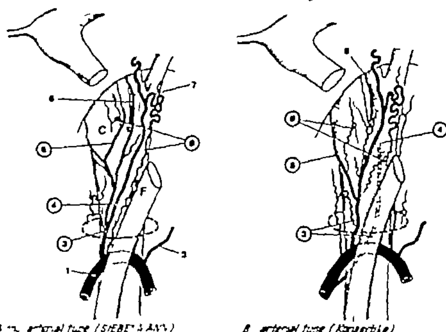


Fig 1 Schematic illustration of the arterial system of the internal auditory canal in man. *1* Anterior inferior cerebellar artery Sub-arcuate artery *3* Labyrinthine artery or arteries. *4* Anterior vestibular artery *5* Cochlear artery *6* Vestibulo-cochlear artery *7* Artery of the vestibular ganglion. *8* Vasa nervorum. The three categories of arteries under investigation are: The labyrinthine artery or arteries (no. *3*), the anterior vestibular artery (no. *4*), the cochlear artery (no. *5*) and the Vasa nervorum (no. *8*).

Table I *Distribution of the degenerative changes observed in the tunica adventitia of vessels with an average outer diameter of 100 μ (arteria cochleae propria or arteria vestibuli anterior) according to decades of life*

Age (years)	No. of cases	Degenerative changes of the tunica adventitia		
		None	Moderate	Severe
0-10	25	25	—	—
11 20	4	4	—	—
21 30	4	4	—	—
31-40	4	2	2	—
41 50	8	1	7	—
51-60	7	—	6	1
61 70	18	—	6	1
71-80	3	—	1	[illegible]
81-90	5	—	1	4
Total	78	36	23	19

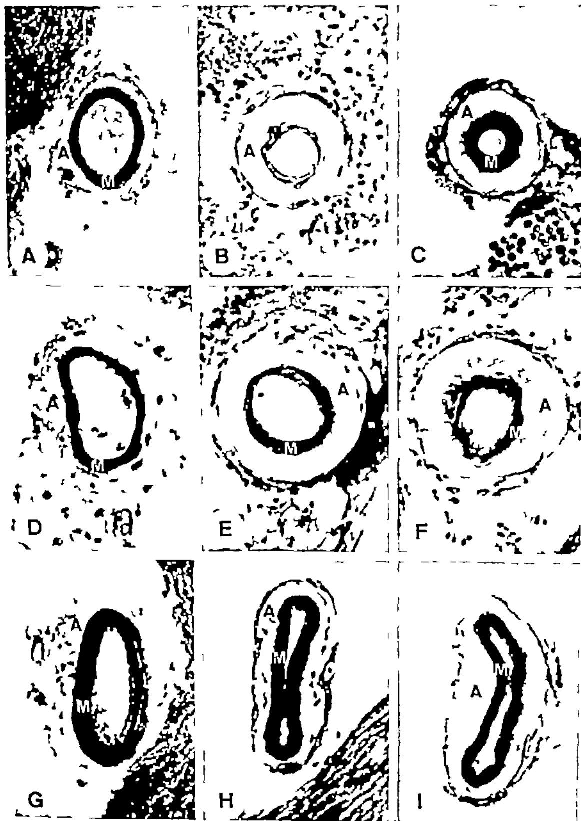
haematoxylin-eosin and Azan. Special stains (PAS, Van Gleson s) could not be used since celloidin-embedded sections already present in the temporal bone collection were used

RESULTS

Normal pattern of the arterial vessels in the internal auditory canal (Fig. 2 *A D G*)

The walls of all three categories of investigated small arteries presented with the three regular vascular layers: The tunica intima, media, and adventitia. The *tunica intima* consists of an endothelial layer surrounded by an internal elastic membrane dividing it from the muscle

Fig 2 Normal histological pattern and degenerative changes of the arterial vessels in the internal auditory meatus (H.E.). Magnification (A F × 400; G I, × 320). (*A B C*) Vasa nervorum in the first, 4th and 7th decade of life (*A* tunica adventitia *M* tunica media). (*D E F*) Cochlear artery in the first, 5th and 7th decade of life (*G H I*). The labyrinthine artery in the first, 5th and 7th decade of life.


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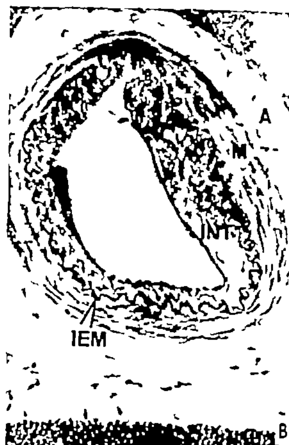


Fig. 3 (*A*) Arteria cochleae propria, 4th decade of life (H.E., ×75). Note the thickening of the internal elastic membrane (*IEM*). *M* tunica media, *A* tunica adventitia. (*B*) Small artery in the region of the geniculate ganglion (H.E., ×320), 7th decade of life. Note the reduction of the lumen due to a thickening of the tunica intima (*INT*). *IEM* Internal elastic membrane; *M* tunica media *A* tunica adventitia.

fibres of the tunica media. The *tunica media* is composed of 2–8 circularly arranged layers of muscle fibres with occasionally interspaced collagenous tissue Elastic fibres were not seen in the tunica media. The *tunica adventitia* is formed by loose strains of connective tissue of variable size containing a few interspaced fibroblasts and surrounded by a thin layer of arachnoidal tissue According to their histological structure the small arteries of the internal auditory canal are of a pure muscular type. They have a thinner wall and less connective tissue than the extracranial vessels of corresponding size Furthermore although the internal elastic membrane is well developed, an external elastic membrane as found in vessels lying outside of the skull is missing

Degenerative changes of the arterial vessels in the internal auditory meatus

(*a*) *Vessels with an average outer diameter of 60 microns (Vasa nervorum)* (Fig. 2 *A B C*). The endothelial layer as well as the internal elastic membrane of the smallest arteries under investigation were not affected by age In no specimen was an increased thickness of the tunica intima observed. By contrast the tunica media loses its compactness during the ageing process and its border line with the adventitia becomes less and less defined (Fig 2 *B*) The most apparent structural modification is seen in the tunica adventitia. This most external vascular layer increases in thickness starting from the first decade of life becoming more and more *fibrotic* The degenerative process

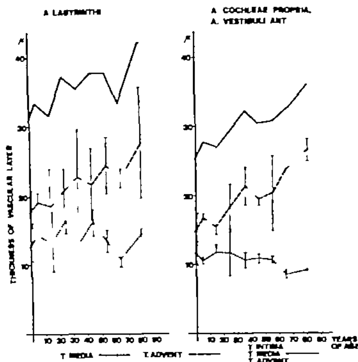


Fig. 4 Thickness of the vascular layer according to the age of the patients. The measurements were obtained using a Zeiss screw micrometer ocular. Note the increasing thickness of the tunica ad entitia, starting from the first decade of life. There is a slight tendency to re duction of the size of the tunica media with increasing age. *I* Standard devia-tion ×2.

of the adventitial tissue is accompanied by a progressive loss of fibroblasts and may finally lead to a total loss of structure (hyalinization). Although the lumen of the Vasa nervorum may show a severe reduction with increasing age (Fig. 2 *C*), a total occlusion of these vessels has rarely been observed.

(*b*) *Vessels with an average outer diameter of 100 microns* (*arteria cochleae propria, ar teria vestibuli anterior*) (Fig. 2 *D E F*) The tunica intima of these vessels as that of the Vasa nervorum is not affected by the in creasing age of the patient The internal elastic membrane may increase in thickness, becom-ing well apparent even with H.E. stain follow ing the third decade of life (Fig. 3 *A*) The tunica media becomes thinner and less com-pact with increasing age (Fig. 2 *E F* and Fig. 4) As in the Vasa nervorum the most striking changes in structures appear with age in the tunica adventitia. The thickness of this layer begins to increase following the first decade of life and after 60 years may reach 60–70% of the original size (Fig. 4) The thickening of the tunica adventitia parallels the appearance of signs of progressive degeneration (increas-ing compactness, loss of fibroblasts and hya linization). These changes are very similar to those occurring in the Vasa nervorum, but develop at a later age, being visible histologic ally only following the third decade of life (Table I) Over 50 years of age no normal vessels of an average outer diameter of 100 microns have been seen and the most severe adventitial changes (loss of structure and acel lularity) become more and more frequent (Table I). The vessels lumen, in contrast to that of the Vasa nervorum, is not affected by the structural changes related with the age of the patients.

(*c*) *Vessels with an average outer diameter of 150 microns* (*labyrinthine artery or arteries*) (Fig 2 *G H I*) The endothelium of the largest investigated vessels did not show any apparent changes with increasing age. The internal elastic membrane, however may be

becoming thicker and showing areas of re duplication. As for the cochlear and vestibular arteries the tunica media became less defined and thinner with increasing age. The same degenerative changes observed in the previous vessels were found in the tunica adventitia. The progressive compactness as well as cellular and structural loss occurring in the most ex ternal vascular layer of the labyrinthine artery are however less pronounced and appear later in time than in the cochlear and vestibular arteries.

A total loss of structure (hyalinization) was rarely observed to occur in the wall of the arteria labyrinthi, even in the oldest age groups.

DISCUSSION

A progressive degeneration of the most ex ternal vascular layer particularly of the tunica adventitia is the most apparent structural change occurring with age in the wall of the arterial vessels of the internal auditory canal. The degenerative changes observed are an increasing thickness and compactness of the ad ventitial tissue followed by a progressive loss of fibroblasts, leading finally to acellularity and loss of structure (hyalinization). Similar degenerative patterns were observed in all three categories of vessels under investigation. The time of onset and the extent of the ad ventitial changes occurring during the aging process were clearly dependent upon the size of the corresponding arteries, the smallest vessels (Vasa nervorum) being involved earlier and to a greater extent than the larger ones (Fig 2) Accordingly a reduction of the vessels lumen was only observed to occur with age in the Vasa nervorum (Fig. 2 *A B C*). The cochlear and vestibular artery as well as the labyrinthine arteries, was found patent and with no definite trend to a narrowing of the lumen up to the ninth decade of life (Fig 2 *D E F G H* and Table I)

The progressive fibrosis and hyalinization found in the adventitial layer of the small arteries of the internal auditory canal with increasing age resemble the degenerative changes observed by Wolkoff (1933) and Baker & Iannone (1959) for smaller intracerebral arteries[1] They are in strict contrast to those observed in arterial vessels of comparative size situated a few millimetres away from the internal auditory meatus in the region of the geniculate ganglion In these vessels (branches of the stylomastoid or petrosal artery) a severe reduction of the lumen due to a thickening of the most internal vascular layer the tunica intima, was found to occur with increasing age (Fig 3 *B*)

One can only speculate about the causes of the progressive degeneration occurring in the tunica adventitia of the arteries of the internal auditory canal with age. One possible explanation could be the particular mechanical stress to which the tunica adventitia is exposed during contraction of the vessels. In fact at surgery following the exposure of the internal auditory canal through the middle cranial fossa for vestibular neurectomy small meatal arteries getting into spasm have been observed to present a reduction of their lumen combined with a little change of their outer diameter Through the operating microscope a contracted small meatal artery appears as a small red line (the lumen filled with blood) surrounded by a thick white wall. This means that the adventitial layer is able to compensate the loss of volume due to the contraction of the tunica media, increasing in thickness. An explanation for this effect which has also been observed to occur in the wall of the smaller arteries of the lung by Hayek (1957)

[1] According to Baker & Iannone smaller arteries are characterized by a size of 190-500 microns The arteries found in the internal auditory canal do not exceed 150-200 microns in diameter and would be considered by Baker & Iannone to be arterioles It is the opinion of most anatomists however that the difference between small arteries and arterioles is not a matter of size but of structure of the tunica media. An arteriole is thus characterized by the presence of a single layer of muscle cells in the tunica media This latter classification was used by the authors in the present study

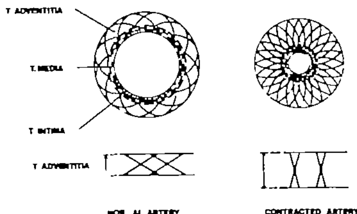


Fig. 5 Schematic illustration of the role of the tunica adventitia during contraction of the small intrameatal arteries. The thickness of the tunica adventitia increases during contraction, whereas the outer diameter of the artery is only slightly decreased.

and in the wall of intra-cerebral vessels by Yasargil (1970) is given in Fig. 5 The particular mechanical stress of the tunica adventitia during contraction could be the reason for the pronounced degenerative changes occurring in this vascular layer with increasing age. Since smaller arteries approaching the size of arterioles are more active and therefore more exposed to stress than larger ones, the mechanical effect would also explain why the degenerative changes occur earlier and are more extensive in the Vasa nervorum than in the arteria cochleae propria or arteria labyrinthi.

Although no functional correlation was attempted in this study one may possibly assume that the degenerative changes of the vascular wall occurring with age may impair the blood supply to the nervous structures of the internal auditory canal and to the inner ear in presence of abnormal hemodynamic conditions. It is in fact well established that the extensibility and contractibility of the vascular wall depend upon the condition of all their elements: muscular tissue elastic and collagenous fibres, as well as of the ground substance (Schallock, 1959) However the changes occurring with age in the vessels of the internal auditory canal are only one of many factors influencing the blood supply to the inner ear structures. All these factors have to be taken into account in order to explain differences in opinion presented in the literature. Although no close relations between the inner ear changes observed with light microscopy and the pathological changes of the basilar artery the anterior inferior cerebellar artery and of the arteries in the internal auditory meatus have been found by Ishii (1967) in a study of 40 temporal bones of aged Japanese people, it cannot be excluded that the adventitial fibrosis may be of functional importance in presence of particular hemodynamic conditions. Pokotilenko (1965) arrived at the same conclusion in studying the temporal bones of 52 patients who died from hypertensive disease

RÉSUMÉ

Les parois des artères du conduit auditif interne montrent, à partir de la première décade, des signes dégénératifs, qui consistent principalement en une augmentation progressive du tissu collagène dans la tunica adventitia. Ces changements peuvent aboutir à la complète hyalinisation de la partie externe de la paroi vasculaire. Les tunica intima et media, par contre, ne montrent pas de modification apparente avec l'âge. Il est possible que les changements observés dans la tunica adventitia des artères du conduit auditif interne en dépendance de l'âge puissent modifier la contractabilité de ces vaisseaux et ainsi limiter leurs fonctions.

ZUSAMMENFASSUNG

Veränderungen sind histologisch in der Wand der arteriellen Gefässe des inneren Gehörganges beobachtet worden. Diese Veränderungen bestehen vor al

in einer Zunahme des kollagenen Bindegewebes in der tunica adventitia sowie in einer hyalinen Umänderung dieser Gefässwandschicht. Die mit zunehmendem Alter beobachteten Veränderungen der Gefässwand könnten die Kontraktibilität der kleinen Arterien im inneren Gehörgang beeinträchtigen und somit zu funktionellen Störungen führen.

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DISCUSSION

B H Colman We have also found bone apposition in the vestibular area but it does not occur as often as in the region of the spiral tract.

We have also found very old people without bone apposition in the region of the spiral tract and who have good hearing according to their case history. I would like to ask Dr Fisch the same question which I put to Madame Nemanić: Has it been possible to examine material from old people with normal or good hearing and how did the vessels appear

Z Krajina. Did you correlate the changes of the auditive artery with the other cerebral arteries in your cases

R Hinchcliffe One recalls the teaching of the otologists at the beginning of the century who referred to presbyacusis as arteriosclerotic deafness. There was also the paper of Fabingi in 1931 the data of which indicated that the degree of hearing loss in elderly subjects was correlated with the degree of degeneration of the intravascular vessels.

J E Bordley Did you make any observations on the stria vascularis and the organ of Corti in these temporal bones and could you correlate these changes with those of the proximal arteries?

U Fisch (Reply) to Mr *Colman* We do not think that the degenerative changes observed in the tunica adventitia with increasing age are the only causes of presbyacusis. We definitely feel however that the increased rigidity of the external vascular layer in combination with other factors, particularly of hemodynamic nature may contribute to presbyacusis

To Mr *Krajina* We have not performed a correlation between the changes observed in the arteries of the internal auditory canal and those situated in the brain. According to the work of Baker & Iannone we have however to conclude that the degenerative changes observed in the meatal arteries are not identical with those observed in intracerebral vessels of corresponding size.

To Mr *Hinchcliffe*: The degenerative changes occurring with age in the small arteries of the internal auditory canal may have some functional implications. Since these changes are mostly *apparent* in the Vasa nervorum the most direct influence of the aging process should be found in the structures supplied by the smallest vessels under investigation.

To Mr *Bordley*: No correlation has been made between the extent of degeneration in the vessels of the internal auditory canal and the stria vascularis

Acta Otolaryng 73 267–269 1972

ELECTRON DIFFRACTION STUDIES ON OTOLITH ORGANIZATION IN THE MACULA UTRICULI OF THE GUINEA PIG

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Abstract The authors have studied the otolith organization in the macula utriculi of the guinea pig. The electronic diffraction patterns obtained from the otolithic membrane and the embedding material are identical, and the same as the amorphous substances. In the otoliths we have found ring diagrams that are neater in the dark zones than in the clear ones. This finding makes us suspect a possible existence of crystalline units in the otoliths that are randomly oriented.

In a previous paper we described (Marco et al., 1971) the fine structure of the otoliths in the macula utriculi. We observed that the otolith bodies are constituted of a dense peripheric area and a clear central one. The extremities are formed by a filamentous-like substance *showing a perpendicular arrangement of the* surface facet.

The purpose of this paper is to provide additional data on the different components of the otolith by means of electronic diffraction.

MATERIAL AND METHODS

The material and methods used have been previously described by us in the Acta Otolaryngologica (Marco et al. 1971)

The samples were examined with a Siemens Elmiskop I electron microscope at 60 kV using an intermediate lens with an aperture of 20 μm.

RESULTS

The electron diffraction patterns obtained from the otolithic membrane and the embedding material (Durcupan ACM) are identical. Both are formed by two concentric haloes as follows: 1) A very diffuse peripheric halo 2) A central halo showing more contrast than the former (Fig. 2 *a*)

Nevertheless, when electron diffraction was performed in the clear areas of the otoliths, a new ring appears whose diameter is less than that of the previous haloes. This *ring arises* with a greater intensity in the diffraction patterns correspondent to the dark areas (Fig. 2 *b c*)

DISCUSSION

Carlström & Engström (1955) studied human otoliths by means of electron microscopy and X-ray diffraction. They indicated that the whole otolith behaves as a single calcite crystal *with the crystallographic c-axis parallel to* their major axis.

Iurato & Petris (1967) obtained spot diagrams from single rat otoliths which are characteristic of single crystals.

To this date, in our microdiffraction studies we have not obtained any spot diagrams which justify the existence of these single crystals.

The diffraction diagrams correspondent to

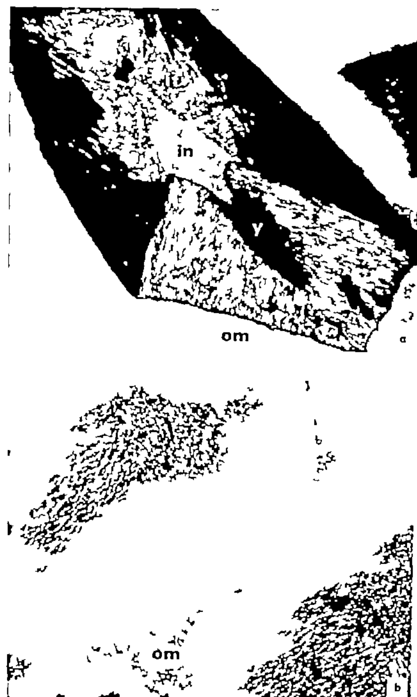


Fig. 1 (*a*) Oblique section of otoliths. In the body of one of them the two components of the central area are clearly distinguished (inner *in*, intermediate *it*) and the dark peripheral area. In the extremity it is possible to detect the filamentous-like substance and the juxtamarginal condensation (*y*). Otolithic membrane (*om*). × 9 000. (*b*) Section of two otoliths. In the extremities the filamentous-like substance is perpendicular to the surface face and in the body is parallel to the main axis of the otolith. × 21 000.

the otolithic membrane and the embedding material (Durcupan ACM) are halo diagrams, characteristic of amorphous substances.

Upon the otoliths we have obtained ring diagrams which are neater in the dark zones than in the clear ones. This makes us suspect the possible existence of small crystalline units randomly oriented. We believe that the existence of these crystalline units and the role that they play in the structure and function of the otoliths is a subject of major interest.

Our aim is to continue the investigation of this problem in order to clearly demonstrate said crystallinity if it exists.

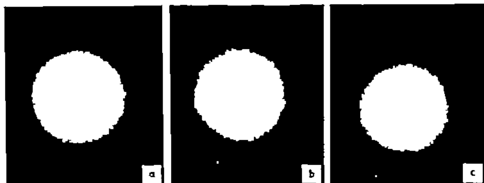


Fig. 2. (a) Electron diffraction pattern obtained from the otolithic membrane and the embedding material. Notice the diffuse peripheric halo and the central one. (b) Electron diffraction pattern obtained from the clear area of the otolith. Observe the appearance of a ring which is placed medial to the halors. (c) Electron diffraction pattern obtained from the dark area of the otolith. The ring is neater than in the clear areas.

RESUMÉ

Nous avons étudié l'organisation des otolithes de la macula utriculaire du cobaye. Le modèle de diffraction électronique obtenú de la membrane otolithique, et du matériel d'inclusion, sont identiques et pareil à ceux des substances amorphes. Dans les otolithes nous avons trouvé des diagrams d'anneaux, qui sont plus net dans les zones obscures que dans les zones claires. Cette découverte nous fait penser à une possible existance d'unites cristallines dans les otolithes orientes à l'hazard.

ZUSAMMENFASSUNG

Die Autoren studierten die Organisation der Otolithen in der Macula utricularis des Meerschweinchens. Das Modell der Elektronendiffraktion, das man aus der otolithischen Membran gewonnen hat, und das Einbettungsmittel, sind identisch und genauso wie amorphe Substanzen. In den Otolithen haben wir Ringendiagramme gefunden, die in den dunklen Zonen deutlicher sind als in den hellen. Dieser Befund lässt uns annehmen, dass möglicherweise kristalline Einheiten — dem Zufall nachorientiert — in den Otolithen bestehen.

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DISCUSSION

S. Iurato. You were very clever in cutting such a hard and difficult material like the otolith. I would like to know if Mr Sánchez-Fernández can tell us something more about the nature of the filaments he demonstrated inside the otoliths, and if the filaments he found between the otoliths have the same size and morphological aspect of those present inside.

J. M. Sánchez-Fernández (Reply) to Mr *Iurato:* About the first question: I think that the filamentous-like substance that I have seen in the otoliths probably corresponds to crystallites as pertains to their different electron diffraction patterns. However I have not studied its chemical composition.

About the second question. I believe that the filamentous-like substance of the otoliths and the fibrillar material of the otolithic membrane are very similar and probably the otoliths are formed within the otolidic membrane by the influence of the ionic component of the utricular endolymph and the content of the macular structures seen by us near the otoliths.

Acta Otolaryng 73 270–279 1972

SUPRAVITAL STUDY OF GUINEA PIG COCHLEA REFERRING ESPECIALLY TO KANAMYCIN LABYRINTHOTOXY

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Abstract. The organ of Corti has been studied supravitally with neutral red vital dye under normal conditions and during kanamycin intoxication. The absorption of the vital dye is described for both circumstances. Normally the living cell absorbs the substance in question as intraprotoplasmic granules, in large numbers. The inner hair cells take up the dye with greater intensity The devitalized cells do not absorb it, or else do so in a diffused manner In animals intoxicated with kanamycin, lack of absorption is observed in the outer hair cells, rarely in the inner ones; furthermore, such phenomena are more pronounced in the lower turns of the cochlea. The reason for this phenomenon is discussed, pointing out the possibility hat it might be due to possible anoxia caused by the g.

With the morphological methods in vogue, from the conventional microscope to the electron all labyrinth images obtained suffer the defect of being static, representing a state of synthesis they display the morphology of what are really cellular corpses. Under pathological and experimental conditions, we can understand only the dynamics of a phenomenon or alteration in a deducible way which is the main limitation of all morphological methods. For some time now we have considered that the vital visualization of the organ of Corti would be highly useful, as a complement to any other type of study since it would give us a dynamic view and would be an authentic physiological experiment

Vital or supravital examination is, on the other hand, the oldest of the histological techniques. Thanks to this method, the histologists of the nineteenth century acquired the first accurate ideas of the structure of the organs and tissues. Such examinations pursue, above all, the study of living cellular elements which have not suffered the action of any physical or chemical agent capable of producing artifacts. Nevertheless, traumatism, anaerobiosis, abrupt suppression of intake, etc., can be the source of new artifacts if the study is not carried out under the most scrupulous conditions. In parasitology biology etc., the field of application of these examinations is large but in human histology it is rarely applied, except in the analysis of hematic elements. However the cochlea is one of the few organs capable of being studied alive: it is small and transparent formed by gracile membranes and few cellular accumulations. Moreover the obtaining of parts of Corti's organ is possible within a short period of time

It appears that Lavdovski (1874) analysed fragments of live cochlea extracted from its osseous envelope but afterwards we find few references until Khlopin (1964) observed the proliferation of cell tissues of the acoustic organ Katsuki & Covell (1953) also extracted fragments but observed them by phase contrast and analysed all their cellular characteristics. Recently it has been Vinnikov & Sokolova (1961) and Vinnikov & Titova (1964) who have been most occupied with the problem.

These authors studied the organ of Corti alive by means of "neutral red" a substance

which is taken up in the form of granules in the protoplasm of the living cell. They further studied cells in a state of relative quiet and after sound stimulation, both in vivo and in vitro They were able to establish that the characteristics of vital absorption vary according to the state of rest or activity a phenomenon which they attribute, in the case of the cochlea, to a certain harmful but unknown action of sound on the cellular proteins. Thus it may be deduced which cells had been in an active state by the distribution of the dye granules in the cell protoplasm.

We too became interested in the problem and began to study during a first phase, the disposition of the acoustic organ by means of supravital techniques, without any treatment, in fresh specimens, but with the use of neutral red, according to Vinnikov & Titova (1964)

We soon abandoned the first phase because of the very great difficulty in obtaining specimens lacking color and consistency and because of the damaging artifacts which we observed. One of the latter was, for example, the presence of cilia having spontaneous rhythmic motion. We then restricted ourselves to the use of neutral red for our vital study The provisional results of this research were presented at the annual meeting of the Spanish Society of Otorhinolaryngology in 1970. Since *then we have embarked on a study of ex*perimental labyrinthotoxy by means of neutral red. Up to now kanamycin intoxication has been investigated, the results of which are described later The ototoxic effect of kanamycin a streptomyces antibiotic discovered in 1957 by Umezawa, was promptly recognized and studied thoroughly as also were other drugs of the group

Communications pointing out this eventuality were immediately presented, both from the clinical and experimental viewpoints (Frost et al. 1958 Lustberg & Hamburger 1959· Naunton & Ward, 1959). The works of Hawkins (1959), Mesolella & Costa (1960), Engström et al. (1964), Kohonen (1965), Koide et al (1966) Saito & Daly (1971), amongst others, have facilitated experimental establishment of many of the characteristics of the functional deficiency produced by kanamycin.

It is known that kanamycin hypoacusia is basically cochlear· that the posterior labyrinth is affected proportionally less than the anterior· that the first cells to be affected are the outer hair cells in the first turn of the cochlea and that, with greater dosages or a longer exposure time, the cells of the other turns are progressively affected. The inner hair cells are more resistant to the toxic action. In sum, the pattern is quite similar to that observed with other streptomyces antibiotics.

The electrophysiological effects of intoxication have been analysed by Hawkins (1959) and Koide et al. (1966) among others Farkashidy et al. (1963) studied the ultrastructure and various histochemical aspects have also been studied.

Kohonen, with Engström's "surface specimen technique"—suitable for the histological study of the intoxicated cochlea—has been able to observe that the degeneration of hair cells follows a definite pattern. As we stated previously the cellular elements of the basal turn are affected first, and the degeneration progresses upward, having previously damaged the internal thread structure of the cells. The *inner hair cells are, in fact, affected less·* but their degeneration begins at the apex and progresses toward the base, i.e. in reverse with respect to the previous, outer hair cells.

This dualism in the behavior of the two types of hair cells has not been definitely clarified, nor why the cellular degeneration continues at a definite rate.

Kohonen, on the other hand by using Maillet's method, has studied the degeneration of the nervous fibres, which never appears in the zones in which the cells are undamaged. This degeneration affects the orthoneurons, while the spironeurons are spared.

On the other hand, it seems that the more sensitive cellular elements are those which are heavily innervated by very granular large

terminations. The writer assumes that the special vulnerability of the outer hair cells of the basal turn corresponds to the greater contribution at these terminations, although this cannot be affirmed categorically

During the present investigation we studied first, the characteristics of the normal cochlea under vital conditions with neutral red and then the results obtained with this technique during kanamycin labyrinthotoxy in order to gain, from any valuable data that may have been obtained, a better knowledge of the mechanism of the ototoxic action of kanamycin.

MATERIAL AND METHOD

Although the number of guinea pigs used was much greater we based this study on 42 animals, 30 of which were used for an analysis of the characteristics of the normal cochlea under vital conditions. The remaining 12 were intoxicated with kanamycin. We used young animals whose weight never exceeded 250 g such animals the osseous structures are le and can be enucleated simply and rapidly with forceps. In this kind of operation, time is important because of the vital nature of the specimen. We also preferred dark colored guinea pigs since they have more deeply pigmented labyrinths, making identification easier

The animals were first examined by otoscopy tonal audiometry from 250 to 4 000 Hz, and Preyer's reflex and turning test. Afterwards, they were decapitated with large scissors without anaesthesia. We proceeded rapidly to disarticulate the jaw and expose the mastoid bullae. Then we extracted the temporal bones, which had to be done as rapidly as possible (one minute may suffice), and placed them in Petri dishes with Ringer solution at 37 C. Under a stereoscopic microscope we opened the bulla and fully exposed the cochlea. The bony shell was chipped away with fine forceps and hooks, exposing about two-thirds of the membranous cochlea in each turn. The spiral ligament was eliminated which sometimes goes along the osseous labyrinth, and Corti's organ remained completely exposed. These operations should be carried out in the quickest possible manner usually being finished in less than a minute Also, generally the second of each pair of temporal bones was processed simultaneously by another member of the team.

The cochleae were immersed in the neutral red solution, rapidly prepared at 0.025% in Ringer solution at 37 C. The samples were placed in the incubator and maintained at 37 C for 45 min, after which they were ready for study

Again with the aid of the stereoscopic microscope and always in the Ringer solution at 37 C, we sectioned each cochlea in two. By this time the neutral red was properly stabilized in Corti's organ and exhibited a deep pink coloration permitting easy identification. The distinct cellular thread structures were observed. There were two masses: one with the first two turns of the cochlear spiral, and the other with the other two

An assistant took charge of one of the blocks and obtained pieces of the four turns of Corti's organ. The solution was replaced periodically in order to keep its temperature close to 37 C. The pieces so obtained were mounted on a slide, covered with a drop of serum and studied under conventional or phase contrast microscopes.

Twelve guinea pigs of the group were submitted to kanamycin treatment with the usual ototoxic dosage Nine of these animals were killed a few days after cessation of Preyer's reflex at 135 dB at all the frequencies investigated. The posterior labyrinth showed hypoexcitability to the turning test, but in no case was it completely absent. The remaining three were killed as described above, before achieving complete loss of Preyer's reflex. Among the three, there was, in one case, lack of response at 4 000 Hz but with normality at the other frequencies. In the other two, not

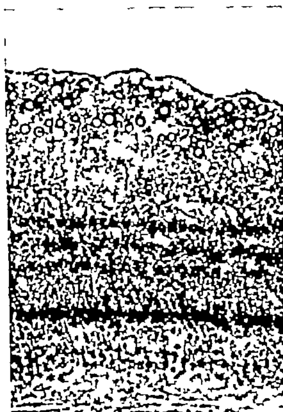


Fig. 1. Apical turn of Corti's organ treated with neutral red, × 22.

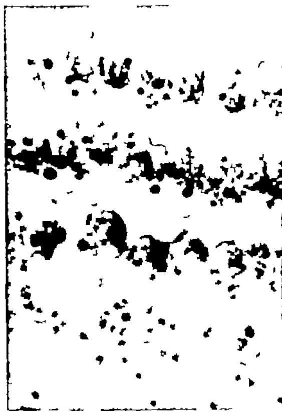


Fig. 2. Outer hair cells of the second turn of Corti's organ. Granules of neutral red may be observed in the cell protoplasm. Immersion objective, × 80.

only was response lacking at 4 000 Hz, but also at 2 000 Hz.

In this manner we had two groups: the first, which can be considered as having injury extending throughout the cochlea (or at least, an ample injury) and the other presumably with partial hypoacusia, with injury in the basal turns. With these last 12 guinea pigs, one temporal bone from each was treated in the manner described, while the other was treated (in an osmium tetroxide solution buffered with Veronal) for a comparative study by phase contrast, according to Engström's method.

RESULTS

From the time in which the cochlea was placed in neutral red, the live cells began to absorb it in the form granules of distinct size which usually varied between 2 and 8 μm and were deposited at a distinct depth in the protoplasm.

Maximum absorption occurred between 30 and 45 min, and the sample started to deteriorate after 1 1/2 hours. With the stereoscopic microscope, Corti's organ was observed heavily stained pink, the distinct cellular thread structures appearing well differentiated. The osmiophilous granules of Hensen's cells appeared bright white and Reissner's membrane was seen stained slightly pink and floating freely.

The spiral ligament and the striae also showed faint coloration. The taking of this type of specimen was more difficult than the osmium-treated samples because of its viscous consistency and the evident difficulty involved in removing the tectorial membrane.

With conventional and phase contrast micro-

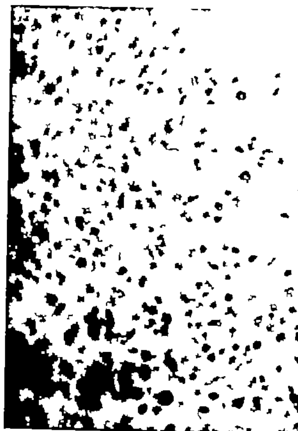


Fig 3 Reissner's membrane. Immersion objective × 80.

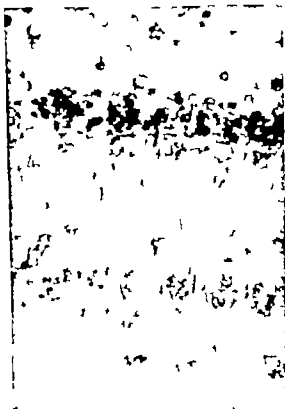
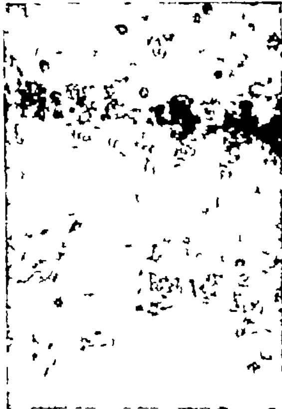


Fig 4 Uptake of neutral red by stria vascularis Immersion objective × 80

scopes, under the conditions described, we could study the characteristics of the vital uptake by the different cellular elements. Since neither staining nor fixing was em ployed, the morphological details were not observed so well as by conventional histological methods. In Fig 1 we see a medium magnified view of a normal Corti organ stained with neutral red a sample pertaining to the apical turn In the lower part of the picture, we observe the inner hair cells with abundant dense and voluminous granules of dye Further along, we observe a zone devoid of granules, corresponding to Corti's pillars. Beyond them, we see the three thread structures of outer hair cells, which exhibit an evident absorption of lesser magnitude than the inner hair cells. On the outside of the outer hair cells, we find those of support Hensen's cells, in which the dye is deposited less densely than in the former though in perfectly individualized granules. The accumulation of lipoids which these cells possess, very numerous in the apical turns, stands out notably

These prepared samples are never sharp because of the distinct depths throughout which the granules of dye are located. It is necessary to vary the focus constantly to locate them. Those that remain out of focus at various depths reduce the image sharpness, since the specimen was of considerable thickness.

In Fig. 2 we have a more magnified view of the outer hair cells. In the sensory cells there is a marked dye absorption of greater magnitude in the inner hair cells, which exhibited thicker granules in their protoplasm, with a confluent tendency The number of granules varies from eight to fifteen. In order to deter

Figs. 5, 6. Two correlated levels of the basal turn of animals intoxicated with kanamycin. Abundant condensation of neutral red is observed in the inner hair cells as well as irregular and deficient absorption in the external ones. Some collapsed external ciliate cells are seen. Immersion objective × 80.

mine with accuracy the cellular zone in which they are located, we stripped some specimens by breaking them, in order to be able to observe the cells longitudinally. We could establish accurately that the staining is deposited in the apical pole of the hair cells in the immediate neighbourhood of the ciliar surface. The same occurs in the supporting cells, although in these it is also observed in the opposite pole. The nuclei were always free of granules.

In Reissner's membrane, as also in the walls of the membranous posterior labyrinth, the disposition of the dye in red spots predominates, sometimes with a central granule, sometimes with a broad empty space corresponding to the nucleus. The granules in Reissner's membrane are usually small, whereas the striae exhibit larger granules while showing a similar disposition (Figs. 3 and 4). The tectoria and the pillars did not take up colorant. Apart from this, there may be observed, above all in the support cells, a certain movement of the abundant intraprotoplasmic organelles. If the specimen is damaged through trauma or is devitalized by cold or anoxia, the cells do not absorb colorant, or do so in a subtle and diffuse manner.

Among the guinea pigs treated with kanamycin the observations were as follows: the cochleae treated with osmic acid displayed a set of characteristics similar to that described by those authors who, before us, studied this intoxication with Engström's surface specimen technique. The damage is particularly prominent in the outer hair cells of the basal turn and diminishes in intensity proportionately towards the higher turns. There are slight

terations in the inner hair cells, always above the second turn.

We have observed a certain lack of correlation between the functional deficiency exhibited by the guinea pig and the damage found. The former was, as a general rule, more pronounced than the latter Those animals that did not demonstrate complete auditory loss, had scarcely any damage and, in one case this was completely absent.

With neutral red we have found two types of phenomena, cellular collapse which is observed more clearly with osmic acid and the lack of stain uptake by normal morphological cells. It is hardly necessary to say that the collapsed cells did not absorb colorant either Between cellular collapse and normal absorption, there is a considerable gradation.

This phenomenon of lack of absorption is the predominant one and is also particularly emphasized in the outer hair cells of the lowest turns. It is almost completely absent in the inner hair cells, which usually exhibit normal characteristics. Only in one case have we observed any of these cells without stain granules. This occurred beginning at the turn, only being able to count up to or four cells which were devoid of it.



Fig 7 Apical turn in animal intoxicated with kanamycin, which conserves sensitivity for the low frequencies. Outer hair cells with good stain absorption. Immersion objective, ×80.

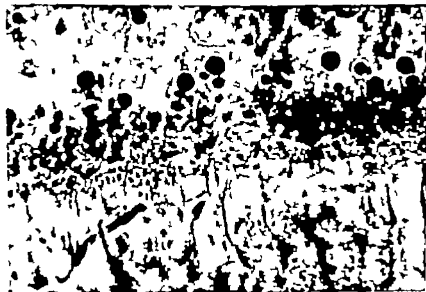


Fig. 8 Inner hair cells, second turn, in which lack of absorption is observed in one of its elements. Immersion objective, ×80.

Figs. 5 and 6 are two correlated views of the basal turn, in which what we stated may be observed clearly: the complete absorption by the inner hair cells and deficiency or absence in the outer ones. These pictures are of a guinea pig intoxicated with kanamycin: the animal had not shown any response at any frequency. Fig. 7 is a view of the surface of Corti's organ, in the apical turn, of a case which responded to 500 and 1 000 Hz; although at high intensities, complete absorption of the colorant is observed in all the cellular elements. Fig. 8 is a microphotograph of the inner hair cells of the second turn, in which we may see the lack of absorption in one cell.

DISCUSSION

We can say little about the normal elements studied, since this was a descriptive research, except to point out the more intense and positive absorption of the dye by the inner hair cells. As regards intoxicated cochleae elements, we detected an outstanding phenomenon: the lack of stain uptake by the cellular elements subjected: this phenomenon is more pronounced in the outer hair cells.

El-Mofty & El Serafy (1966) and Mizukoschi & Daly (1967) demonstrated that kanamycin, as well as other ototoxic antibiotics, impede the normal intake of oxygen by the cochlea. Ilberg et al. (1971) by autoradiographic means, demonstrated that there probably exists a special susceptibility to streptomycin of the hair cells which establishes a reversible pattern in the cellular ribosomes, as also occurs with bacteria. In addition, they showed that the specific sensitivity of the sensory cells of Corti's organ would have to be sought in the long persistence of the antibiotic in the perilymph. Ostin & Tyberghein (1968) and Voldřich (1965) said the same also.

The lack of absorption of the vital dye in our cases, was quite similar to that which would have taken place during a situation of anoxia. In the cases studied, we could discount traumatism and chill, since all the specimens examined were ideal—those that were not, were excluded. With other causes discounted, anoxia was probably the only factor responsible for the lack of uptake.

There exist studies, now old (Umbreit et al., 1949), which indicate the important role of streptomycin as an element capable of interfering in the systems of cellular respiration, both in bacteria and in tissue. We presumed that kanamycin would be similar, as a drug of the same group.

Our observations strongly suggest an anoxic mechanism to be involved in the cochlear damage, because of the lack of absorption of vital colorant. We are currently carrying out an investigation of the possible ototoxicity of gentamycin. So far, we have obtained damage only to the posterior labyrinth. Nevertheless, if we ligated a ureter, before there was a possibility of compensation, some hypoacusia was produced. The ligation of a ureter assumes a transient renal insufficiency and therefore the insufficient elimination of the drug. In such a case, Corti's organ was exposed to higher and more prolonged concentration of the drug and damage resulted. With the barrier that the labyrinth usually offers overcome, the persistence factor should be fundamental in explaining the ototoxic action of some antibiotics. In effect, Corti's organ does not have a vascularization similar to the distinct organic tracts. It receives its intake indirectly across the endolymph (or cortilymph), instead of directly from the vascular system. The noxious molecules much accumulate in the fluid thus favoring an ototoxic action due to longer contact.

The gentamycin should doubtless be a less ototoxic antibiotic; but when we increased its concentration by means of ureteral ligation it revealed its ototoxic potentiality. The application *in situ* of any ototoxic has always been particularly harmful for the same reason, increased concentration and persistence of the drug.

Notwithstanding the special predilection of

some antibiotics with respect to Corti's organ we believe that its peculiar nutritional require ments via fluids with essentially slow re-ex change is a very important factor in the pathogenesis of any ototoxic antibiotic and that this undoubtedly interferes with cellular respiration.

CONCLUSIONS

1) The vital dye is accumulated in the protoplasm of the living cells of Corti's organ in the form of intraprotoplasmic granules of distinct size and number The peak penetra tion is attained after 45 minutes of exposure.
2) Practically no granules are observed in the cellular nuclei.
3) The absorption is more intense in the inner hair cells, less so in the external ones, and though in lesser proportion it is localized in the support cells.
4) The vascular stria and Reissner's membrane exhibit typical and considerable absorption.
5) The pillars and the tectorial membrane show no absorption.
6) The devitalized cells absorb no dye, or do so in a diffused manner
7) The cells affected during kanamycin intoxication do not absorb the stain.
8) This phenomenon is observed more frequently in the outer hair cells than in the inner ones.

RÉSUMÉ

Nous avons étudié la morphologie de l'organe de Corti vivant, ainsi que l'absorption d'un colorant vital (rouge neutre). Nous avons utilisé le microscope ordinaire et le microscope à contraste de phases. Dans la deuxième partie du travail nous avons étudié des animaux traités par la Kanamicine et la Gentamicine.

ZUSAMMENFASSUNG

Das Cortiorgan wurde über das Leben hinaus mit neutralem Rot studiert, sowohl unter normalen Bedingungen als auch bei Vergiftung mit Kanamicin. Es wurde die Annahme des vitalen Farbstoffes in beiden Zuständen beschrieben. Normalerweise absorbiert die lebende Zelle die sich im Studium befindliche Substanz als intraprotoplasmatisches Körnchen und in verschiedener Anzahl.

Die inneren verästelten Zellen nehmen den Farbstoff stärker an, die nicht lebenden Zellen nehmen ihn nicht an oder nur verschiedentlich aus welchem Grund es immer sein möge.

Bei Tieren, die mit Kanamicin vergiftet wurden, beobachtet man die Annahmeunfähigkeit bei den äusseren verästelten Zellen, selten bei den inneren. Diese Erscheinung tritt stärker in den unteren Schichten der Schnecke auf

Es wird der Grund dieser Erscheinung diskutiert, und man nimmt an, dass dies auf einen möglichen Sauerstoffmangel zurückzuführen ist, der durch die Droge entstanden ist.

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Acta Otolaryng 73 280-289 1972

THE EFFECT OF SODIUM-CYANIDE ON THE CHICK EMBRYO OTOCYST IN VITRO

A Tissue Culture Study

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Abstract Certain neurogenic degenerative diseases in Nigeria are associated with optic atrophy and perceptive deafness. It has been suggested that a high dietary cyanide intake in a malnourished population might be a contributory factor In the present studies organ cultures of the fowl embryo otocyst were exposed to various concentrations of sodium cyanide and the ensuing degenerative changes on nerve tissue and other cells are described.

Nutritional neuropathies are common in certain tropical countries such as Nigeria, Jamaica (Hinchcliffe & Mrall, 1965) and elsewhere. Monekosso & Wilson (1966) have described ~ clinical findings of nutritional ataxic neu in Nigerian patients. This is a neurogenic degenerative disease characterized by a predominantly posterior column myelopathy with or without peripheral neuropathy associated with bilateral optic atrophy and bi lateral perceptive deafness. The natural history of the disease is one of slow progression Williams & Osuntokun (1969) encountered no fatal cases among their patients. The aetiology is not known but it has been suggested that a high dietary cyanide intake in a malnourished population might be a contributory factor

Williams & Osuntokun (1969) have described the light microscopic features of the peripheral nerves from 23 Nigerian patients and the electron microscope findings in the peripheral nerves from 4 of these patients.

The research has been aided by grants from the Wellcome Research Foundation and the Cancer Campaign for Research.

The light microscopic changes were consistent with segmental demyelinization and, under the electron microscope, these were characterized by "disintegration and lysis of myelin lamellae and/or myelin digestion in a patchy fashion" These findings, although "not specific" were also observed by Williams & Osuntokun (1969) in rats a few weeks after injection of a single dose of potassium cyanide.

Further experimental investigations seemed to be indicated with particular reference to the effect of cyanide on the tissues of the developing inner ear

Otocyst organ cultures are eminently suitable for the study of toxic agents acting upon the constituent tissues of the cultured otocyst: "a model ear" as was shown by Friedmann & Bird (1961) and Friedmann (1965). We have described the in vitro development of innervation of the inner ear (Friedmann, 1969) and the high differentiation of its neurones, the formation of non myelinated and myelinated nerves and of the neurojunctional apparatus.

The present report describes the effect of a cyanide compound on the neurogenic elements and other cells of the cultured chick embryo otocyst.

MATERIAL

The isolated chick embryo otocysts (for techniques see Friedmann & Bird, 1961 Friedmann, 1969) were exposed to sterile sodium cyanide



Fig. 1 EM. 11232. Sensory epithelium. There is bulging of the surface cell membrane and there are many phagosomes in the cytoplasm. Note: preserved mitochondria and cilia. (100 µg sodium cyanide.) 10 800.



Fig. 2. EM. 11226. Note rudimentary kinocilium on the surface of a supporting cell. Also stereocilia and crosscut kinocilium of a deeper hair cell. (100 μg sodium cyanide.) × 28 400.



Fig. 3. EM. 11200. Detail of undifferentiated, probably supporting cell. Note primary cilium and basal corpuscle in the vacuolated cytoplasm (near the nucleus, not included at this magnification of the cell). (100 μg sodium cyanide.) 71 300



Fig. 4. E.M. 11399. Degenerating nerve axon with myelin figures. (50 μg sodium cyanide.) × 32 500.



Fig. 5. EM. 11297. Two healthy neurones (control) 5400.



Fig. 6. EM. 11305 Degenerated neurone. (100 μg sodium cyanide.) × 5 400.



Fig. 7 EM. 11313. Severely damaged neurone. (200 µg sodium cyanide.) × 6 780.

in varying concentrations, applied at the time of the final transfer of the developed cultures: i.e 48 hours before completing the culture at an overall age of the developing otocyst of about 16 days. Three concentrations of sodium cyanide were used, 50 100, and 200 μg per ml media.

RESULTS

The results can best be summarized according to the tissues affected

Neuroepithelium

There was widespread damage of the cell membranes in particular of the outer cell membrane on the surface of hair cells which had been weakened by the poison resulting in extensive bulging of the outer cell membrane (Fig. 1). There was greatly enhanced lysosomal activity leading to the formation of multicystic phagosomes in many of the sensory cells (Figs. 1 2) It is interesting to note that at the concentrations applied most of the mitochondria appeared to be well preserved (Figs. 1 2)

Ciliary apparatus

In spite of the obvious changes both in the cell and in its innervation (see below) many of the ciliary structures seem to have survived. This applies especially to the primary or rudimentary kinocilia sprouting from various sensory and also supporting cells (Figs. 2, 3)

Nerve fibres

There was in all the concentrations applied, widespread degeneration of the developing myelinated and non myelinated nerve fibres, and there were many myelin figures. Fig 4 compares well with the illustrations in the paper by Williams et al. describing peripheral nerve changes in their Nigerian patients. (These changes are not specific and may occur in various segmental neuropathies, Williams & Osuntokun, 1969.)

Neurones

Fig 5 shows two healthy neurones in a control culture of filamentous and granular type with surrounding satellite cells. Figs. 6 and 7 show the effect of sodium cyanide on various neurones, most marked in the higher concentrations applied resulting in the partial to complete dissolution of the cytoplasm of the cells (Figs. 6 7)

DISCUSSION

The present study reports the results of the experimental application of a biological system in the investigation of a practical neurological problem of the inner ear Organ cultures of the isolated chick embryo otocyst have been exposed to the effect of varying concentrations of sodium cyanide which caused widespread degeneration of nerve tissue including the neurones of the developing inner ear

This effect not mentioned by previous authors might explain many of the clinical signs and symptoms of central nerve system origin noted in Nigerian patients by Monekosso & Wilson (1966). Neuroepithelial cells suffered degenerative changes. Their cell walls especially the outer cell membrane was damaged in the same way as in organ cultures or in tissues exposed to various ototoxic antibiotics.

Another feature worth mentioning was the comparative resistance of the ancillary apparatus of the hair cells. The cilia have suffered less than might have been expected. Rudimentary and primary cilia have shown considerable resistance and it may be assumed that some of the cells might have in fact developed new rudimentary cilia. Such structures were first described by us in the developing otocyst and have been elevated to an important role in the proper functioning of the hair cell. The resistance of these structures may be interpreted as evidence of their importance although they would be unable to function following the damage of other links of the functional chain hair cells, nerves and

neurones, eliminated or paralysed by a potent cell poison such as cyanide

ACKNOWLEDGMENTS

My sincere thanks are due to my collaborator and chief technician E. S. Bird, F.I.M.L.T. and to Miss Jacqueline Attan for the excellent electron photomicrographs.

RÉSUMÉ

Certain neuropathies en Nigerie sont accompagnées de surdité et d'affaiblissement de vue. On soupçonne une intoxication chronique par le cyanide dans la nourriture de la population. Des cultures de vésicules auditives de poulets ont été exposées à l'action de cyanide du sodium. Les lésions des nerfs et des cellules nerveuses, ainsi que de l'épithélium sont décrites

ZUSAMMENFASSUNG

Gewisse degenerative Neuropathien die in Nigeria vorkommen, sind durch eine optische Atrophie und Stummheit gekennzeichnet. Es wird angenommen, dass die Ursache in einer chronischen Cyanid-vergiftung ihren Ursprung hatte und seit der Ernahrung zusammenhänge. Otocyst-Organ-kulturen wurden in vitro der Wirkung von Natrium-Cyanid ausgesetzt. Die Befunde am Sinnes-epithelium und insbesondere an Nerven und Ganglienzellen werden beschrieben.

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DISCUSSION

R. Hinchcliffe: I would like to emphasize the value of Mr Fernández studies in the sense that these tropical oto-neuro-ophthalmological syndromes are of considerable practical importance, existing in very high prevalence in some tropical regions. There is no doubt that in Nigeria the condition is associated with the consumption of large quantities of cassava. Cassava contains a cyanogenetic glucoside, linamarin. In Senegal a similar neuromyelopathy exists but here the staple diet is millet, which contains the cyanogenetic glucoside dhurrin. However it seems unlikely that a chronic cyanide poisoning is responsible for the neuromyelopathic syndrome in Jamaica since first, little if any cassava or millet is consumed there, and secondly there are no changes in blood chemistry that characterize chronic cyanide poisoning.

R. Thalmann: The isolated otocyst can serve as an excellent model for a variety of biochemical experiments which could never be carried out in the whole animal. We must thank Prof. Friedmann for his pioneering efforts in this field. It would be very interesting to study the metabolic changes occurring as a consequence of cyanide intoxication using quantitative microchemical techniques similar to those used in the mammalian inner ear. In the case of the otocyst it would of course be necessary to section the tissues in the frozen state prior to freeze-drying. I would like to ask Prof. Friedmann whether it is possible to establish cultures of single cell lines, for instance of hair cells. If this were possible a variety of *in vitro* experiments in common use in cell biology would become possible and sufficient material might be available for differential ultra-centrifugation in order to study separate subcellular fractions.

C. Fernández: I have studied the action of osmium tetroxide on the tracheal ciliated cells of rat and I have observed that the ciliary regenerative processes take place in most of epithelial cells during the 7 days following the action of toxic vapours. Such processes are identical with ciliogenesis in the tracheal epithelium during fetal life. My question is: do you think that the ciliogenesis consequent upon the toxic effect of cyanide and other toxin is similar?

I. Friedmann (Reply) to Mr *Hinchcliffe*: You have rightly raised the question of the differences in the causation of the various tropical neuropathies. I thank you for underlining the useful application of our methods in an attempt to try and find an answer experimentally to this problem of great practical significance.

To Mr *Thalmann*: You have opened up a great vista of research on single cell lines from the neuroepithelium. I think it possible that such cell lines could be established.

To Mr *Fernández*: I agree that the ciliogenesis in the labyrinth does not seem to differ fundamentally from that of the ciliated epithelia elsewhere. Toxic agents can encourage the development of primary cilia from the pre-existing centriole of apparently any cell.

Acta Otolaryng 73 290-295 1972

ÜBER DIE KIEFERHÖHLENFONTANELLE

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Abstrakt Nach kurzer Besprechung der Anatomie, Histologie und der eventuellen Funktion der Nasenfontanellen werden ihre in der Literatur bisher bekannten Veränderungen zitiert. Mit der Nasenendoskopie können die Fontanellen heute gut überblickt werden. Bei der akuten Sinusitis maxillaris sind sie entzündlich vorgewölbt, pulsieren im Ganzen und füllen den ihnen entsprechenden Teil des mittleren Nasenganges aus. Bei der chronischen Entzündung sind die vorgewölbt keilförmig oder zitzenartig, wiederholt mit Pigmenteinlagerungen in der Schleimhaut. Dazu kann es zu Abszeßbildung, Konkrementbildung in einer Tasche oder zum Vorstülpen eines „Choanalpolypen" aus einem accessorischen Ostium u. a. kommen. Bei der chronischen Rhinitis können durch Hyperplasie des nasalen Schleimhautblattes keil- oder leistenartige Fontanellenformen entstehen; außerdem kann von einer nicht seltenen Schleimhautmetaplasie Bereich der mittleren Fontanelle einmal ein Kiefer teatom ausgehen. Auch maligne Tu- können on der Pars membranacea aus Abschließend wird überlegt, ob die Nasen- skopie es heute gestattet, verschiedene Erkrankungen, so wie schon früher versucht, durch endonasale Eingriffe unter Führung der Nasenoptiken zu behandeln.

Zwischen den Fortsätzen des Processus uncinatus und denen des Os turbinale bzw dem oberen Anteil der Lamina perpendicularis des Os palatinum fehlt in verschiedenem Ausmaß der Knochen. In diesem Abschnitt ist die laterale Wand des mittleren Nasenganges also membranös und Nasen und Kieferhöhlenschleimhaut liegen direkt aneinander Zuckerkandl (1893) hat diese Abschnitte als Nasenfontanellen bezeichnet, während Hajek (1926) von einer Pars membranacea des mittleren Nasenganges spricht Terracol & Ardouin (1965) bezeichnen diese Abschnitte je nach ihrer Lokalisation als vordere, mittlere oder hintere Fontanelle. Onodi (1906) hat die verschiedene Ausdehnung dieser membranösen Partien in seinen anatomischen Tafeln vortrefflich demonstriert.

Histologisch bildet die Basis dieser membranösen Bezirke ein vom Periost der begrenzenden Knochen ausgehendes feines Fasernetz, an das sich die Nasen- und Kieferhöhlenschleimhaut anlagert. Knapp über dem Fasernetz findet man auf beiden Seiten relativ starke Arterien, kavernöse Räume und starke Äste von marklosen Nervenfasern, die in engster Beziehung mit den Gefässen verlaufen.

Ob diese regelmäßig vorkommenden, in ihrer Ausdehnung aber sehr variablen Fontanellen eine Funktion haben oder nur zufällige Bildungen der Natur sind, läßt sich nicht abschätzen solange die Funktion der Nebenhöhlen nicht bekannt ist. Ich konnte jedenfalls bei der Kieferhöhlenendoskopie und forzierter Nasenatmung an normalen Fontanellen geringe Ein- und Auswärtsbewegungen beobachten die bei schnaubender Exspiration flatternd wurden. Dies könnte vermuten lassen, daß die membranöse Kieferhöhlenwand zusammen mit dem Ostium dieselbe Aufgabe hat wie der den Druck steuernde Teil einer Wilsonschen Nebelkammer nur läßt sich dies bisher nicht beweisen.

Die Fontanellen können natürlich allein erkranken von pathologischen Prozessen ihrer Umgebung irritiert werden bzw an ihnen teilnehmen. So hat Hartmann als Erster eine Vorwölbung der Fontanelle des mittleren Nasenganges bei einem Kieferhöhlenempyem

konstatiert, die angeblich nach der Spülung verschwand. Auch Hajek (1926) Kasparlantz, Marx (1949) Ziem (1886) u.a. haben fallweise solche Protrusionen beobachtet und sie vorerst wie Marx (1949) als Folge einer Eiterretention gedeutet. Hajek (1926) allerdings hielt es nicht für ausgeschlossen, daß dafür nicht einmal ein vollkommener Verschluß des Kieferhöhlen ostiums notwendig sei es dürfte seiner Mei nung nach für ihr Entstehen schon die relative Behinderung des Abflusses durch das Ostium maxillare genügen. Daß dieses Symptom keine großere Bedeutung erlangt hat und heute fast in Vergessenheit geraten ist, dürfte wohl darin liegen daß bei der Rhinoskopie es nicht im mer leicht ist festzustellen ob die laterale Nasenwand vorgewölbt ist, es ist ja auch unter normalen Verhältnissen der mittlere Nasengang sehr verschieden tief so daß die Grenze zwischen normalen Verhältnissen und einer geringfügigen Hervorbuchtung der Fontanelle gegen die Nasenhöhle bei der Rhinoskopie schwer zu ziehen ist.

Mit der Rhinoskopie konnte man bisher nur einen sehr begrenzten Einblick in den mitt leren Nasengang erzielen. Nimmt man aber Endoskopieoptiken zu Hilfe, dann kann man tief in die verschiedenen Nasengänge eindringen und sie eingehend untersuchen (Messerklinger 1970) Besonders leicht, und prak tisch in jedem Fall ist dabei die Gegend der Kieferhohlenfontanelle zu überblicken. Ich untersuche am liegenden Patienten in Ober flächenanaesthesie mit Hopkinsoptiken, 4 mm stark, mit einem Blickwinkel von 30 und 70 Grad und geradeaus die einzelnen Nasengänge von rückwärts nach vorne dieser Modus ist deswegen notwendig, weil die Nasengänge rückwärts weit sind, dadurch das Eindringen einer Optik ohne weiteres gestatten und man dann ohne Schleimhautläsion, die Muscheln abdrängend, die Optik bis in den vorderen Anteil der einzelnen Nasengänge vorziehen kann

Für die Untersuchung der membranösen Partien im mittleren Nasengang sind vor allem die mittlere und die hintere Fontanelle interessant während eine vordere eine ausgesprochene Rarität ist. Die hintere Fontanelle kann vom Ende des mittleren Nasenganges nach vorne bis fast an das Kieferhöhlenostium und darüber und darunter reichen, während die mittlere unter der hinteren Hälfte des Proces sus uncinatus liegt, und bei extremer Ausbildung den Raum vom oberen Rand des Schwellkörpers der unteren Muschel bis fast zum freien Rand des Processus uncinatus ein nehmen kann.

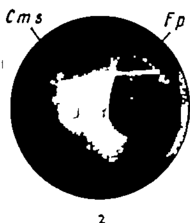
Bei der endoskopischen Untersuchung der Fontanellegegend kann man nun folgendes beobachten.

Relativ oft findet sich ein accessorisches Kieferhöhlenostium, nach der Statistik Zuc kerkandls (1893) in jedem zehnten Fall. Es ist vorwiegend die mittlere Fontanelle in der sich diese Ostien finden (Abb 1) während sie in der hinteren selten sind (Abb 2) Die Grösse der Ostien schwankt von Öffnungen, die mit dem Endoskop gerade sichtbar sind bis zu solchen, die einen guten Einblick in die Kiefer höhle gestatten. Aus solchen Ostien kann oedematöse Kieferhöhlenschleimhaut in den mittleren Nasengang prolabieren und zu einem großen „Choanalpolyp" werden (Abb 3) In solchen Fällen findet man bei der Kiefer höhlenendoskopie einen wesentlichen Teil der medialen Kieferhöhle mit oedematöser zum Teil auch zystisch durchsetzter Schleimhaut ausgefüllt, während die übrige Mucosa prak tisch normal sein kann.

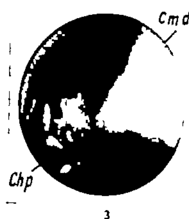
An einer akuten Sinusitis maxillaris nimmt natürlich auch die Pars membranacea teil sie wird entzündlich infiltriert und dadurch mit oder ohne Verschluß des Kieferhöhlenostiums gegen das Lumen des mittleren Nasenganges vorgewölbt Ist eine große mittlere Fontanelle vorhanden dann füllt ein akut entzündlich geröteter und pulsierender Schleimhautprolaps, der fast bis zum Schwellkörper der unteren Muschel reichen kann, die mittlere Partie des mittleren Nasenganges aus (Abb. 4) Charak teristisch für diese extreme Fontanellenvor wölbung ist es, daß sich die Kieferhöhle vom unteren Nasengang aus nicht oder nur sehr schwer spülen läßt dies kann zwanglos da



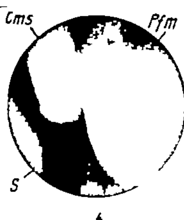
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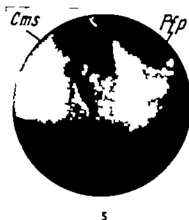
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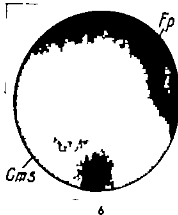
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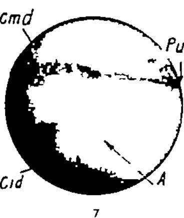
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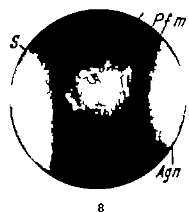
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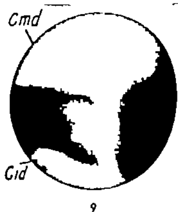
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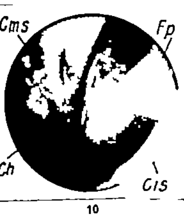
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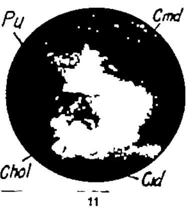
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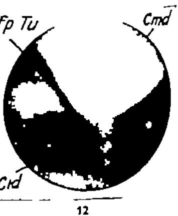
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12

durch erklärt werden, daß auch die sehr schwellungsfähige Schleimhaut des Processus uncinatus an der Entzündung teilnimmt und zusammen mit dem Fontanellenprolaps das Kieferhöhlenostium maximal einengt. Bildet sich die Vorwölbung zurück, dann gelingt auch die Kieferhöhlenspülung wieder leicht. — Eine hintere Fontanelle reagiert auf eine akute Sinusitis maxillaris ebenfalls mit einer extremen Vorwölbung in ihrem Bereich (Abb 5) und füllt das normalerweise weite Gewölbe des hinteren Drittels des mittleren Nasenganges aus (Abb 6) Ihre Schleimhaut ist ebenfalls akut gerötet und die ganze Vorwölbung pulsiert. In diesen Fällen kann man oft auch das Kieferhöhlenostium sehen, das durch die entzündete Mucosa meist schlitzförmig eingeengt ist Schwierigkeiten bei der Kieferhöhlenspülung habe ich bei hinteren Fontanellenprolapsen nicht erlebt. — Klingt die akute Entzündung ab dann bildet sich auch die Vorwölbung der Pars membranacea zurück es verschwindet zuerst die pralle Füllung des Prolapses, die Protrusion wird oft keilförmig und die Schleimhaut darüber regelrecht runzelig Auch wenn kein pathologisches Sekret mehr ausgespült werden kann und kein Stenosegeräusch vorhanden ist, vergehen bis zur Normalisierung der Fontanelle meist doch einige Wochen

Bei der chronischen Sinusitis maxillaris kann der Zustand der Fontanellen recht vielfältig sein: meist sind sie durch chronisch-entzündliche Schleimhautinfiltration vorgewölbt, keilförmig oder auch zitzenartig es fehlt aber die pralle und extreme Protrusion und Pulsation der akuten Entzündung. Die histologischen Veränderungen solcher Fontanellen reichen von der chronisch-entzündlichen Infiltration über die haemorrhagische Infarcierung, über eine Abszedierung (Abb 7 = Abszeß in einer rechten mittleren Fontanelle) bis zur narbigen Induration so kann z. B aus einer zitzenartigen Vorwölbung ein pendelndes Fibrom entstehen. In einem Prolaps der mittleren Fontanelle können sich auch Konkremente bilden dies führt zusammen mit der chronischen Entzündung und ihren akuten Exacerbationen zu einer dauernden Vergrösserung des Prolapses, der schließlich nur mehr nach vorne in den mittleren Nasengang einen Ausweg findet, den Kopf der mittleren Muschel überlagert und aussieht wie eine erkrankte Concha bullosa (Abb 8). Im Röntgenbild stellt sich das Kon-

Abb 1 Accessorisches Kieferhöhlenostium in einer mittleren linken Fontanelle.

Abb 2. Accessorisches Kieferhöhlenostium in einer linken hinteren Fontanelle.

Abb 3 „Choanalpolyp aus einem accessorischen Ostium der rechten mittleren Fontanelle kommend.

Abb 4 Prolaps einer linken mittleren Fontanelle bei akuter Sinusitis maxillaris.

Abb 5. Prolaps einer hinteren Fontanelle bei akuter Sinusitis maxillaris links, den hintersten Teil des mittleren Nasenganges ausfüllend.

Abb 6. Normaler kuppelartiger mittlerer Nasengang links, hintere Hälfte.

Abb 7 Absceß in der rechten mittleren Fontanelle.

Abb 8 Ein ein Konkrement enthaltender Prolaps der linken mittleren Fontanelle, der einen mittleren Muschelkopf vortäuscht.

Abb 9 Rechte mittlere Fontanelle bei chronischer Sinusitis maxillaris mit eingeengtem, accessorischem Ostium und Pigmenteinlagerungen

Abb 10 Linke hintere Fontanelle mit Leistenbildung bei chronischer Rhinitis.

Abb 11 Rechte mittlere Fontanelle mit einer Perforation aus der Cholesteatommassen ragen.

Abb 12. Rechte hintere Fontanelle, derb-höckerig infiltriert und den mittleren Nasengang ausfüllend — Retothelsarkom.

Abkürzungen

A = Abszeß
a O = accessorisches Ostium
A g n = Agger nasi
Ch = Choane
Ch p = Choanalpolyp
Chol = Cholesteatom
C i d = Concha inf dextra
C i s = Concha inf. sinistra
C m d = Concha med. dextra
C m s = Concha med. sinistra
F p = Fontanella post.
P u = Processus uncinatus
P f m = Prolapsus fontanellae med.
P f p = Prolapsus fontanellae post.
S = Septum

krement direkt neben dem Septum dar. kennt man diesen Zustand nicht, sucht man vergeblich nach dem Fremdkörper in der Nase. Dieser Befund dürfte keine all zu große Rarität sein, denn ich konnte im letzten Jahr zwei solche völlig gleichartige Fälle operieren. — In der Fontanellenmucosa findet man bei der chronischen Entzündung auch wiederholt zarte bräunliche Pigmentflecke wahrscheinlich Haemosiderinablagerungen (Abb 9) Die accessorischen Ostien sind durch die chronische Entzündung sichtlich verkleinert (Abb 9)

Wie die Fontanelle an Erkrankungen der Kieferhöhlenschleimhaut teilnimmt, wird sie auch in pathologische Zustände der Nasenhaupthöhle einbezogen. Eine chronische Rhinitis bedingt so als Ausdruck einer ausschließlichen oder vorwiegenden Erkrankung des nasalen Mucosateils eine keilförmige Fontanellenform oder eine Leistenbildung (Abb 10) Die Schleimhaut der Gegend der mittleren Fontanelle ist anscheinend wesentlichen Irritationen durch den Luftstrom mit seinen Beimengungen ausgesetzt man findet gerade in diesem Areal bei sonst normalem respiratorischem Epithel ausgedehnte Epithelmetasen. Von diesem Bezirk aus kann sich durch eine Vertiefung oder auch durch ein kleines Ostium ein Cholesteatom in die Kieferhöhle entwickeln, wie Abb 11 zeigt. Bei diesem Fall sah man im Röntgenschichtbild von der mittleren Fontanelle ausgehend eine dem Cholesteatom entsprechende Verschattung, die die Höhle großteils ausfüllte, ihre Randpartien aber frei ließ Die Kieferhöhlenschleimhaut war chronisch entzündet, hyperplastisch und mit mehrreihigem Flimmerepithel besetzt.

Bei Verletzungen kommt es durch collaterale Schleimhautschwellungen und die Blutung in den Sinus zu keilförmigen Fontanellen dazu ziehen meist Blutkoagula, die 10 bis 14 Tage liegen bleiben können, aus Ostien über die Fontanellengegend und konnen so wertvolle Hinweise auf die Lokalisation der Fraktur geben.

Auch maligne Neubildungen können von einer Fontanelle ausgehen. Wie Abbildung 12 im Falle eines von der Pars membranacea entspringenden Retothelsarkoms zeigt, ist die Fontanelle dabei derb-höckerig infiltriert und füllt die entsprechende Partie des mittleren Nasenganges aus.

Allein durch die endoskopische Untersuchung der Nasenfontanelle lassen sich also wertvolle Hinweise für Diagnose und Therapie gewinnen. Damit erhebt sich aber auch die Frage, ob wir nicht heute unter wesentlich günstigeren Bedingungen in der Therapie nochmals Wege versuchen sollen, die man vor über einem halben Jahrhundert gegangen ist und die jetzt praktisch ganz verlassen sind. Zuckerkandl (1893) hat ja schon vor über 70 Jahren darauf hingewiesen daß der mittlere Nasengang durch seine ausgedehnte membranöse Beschaffenheit sich besonders gut für die Eröffnung der Kieferhöhle eigne. Ausser zur Spülung der Kieferhöhle mit verschiedenen Röhrchen (Hartmann Siebenmann, Weil, Dencker v Eicken u. a.) hat Siebenmann (1912) als Erster den Sinus maxillaris durch die Fontanelle breit eröffnet, indem er zuerst mit einem scharfen Löffel perforierte und dann die Pars membranacea mit Schlinge und Stanze resezierte Killian und Boenninghaus (1913) schnitten die Fontanelle mit dem Sichelmesser aus, Bayer benützte dazu den Galvanokauter Onodi (1906) gab einen eigenen Troikart für Punktion und Dilatation der Fontanelle an, während Wagner sie einfach mit dem kleinen Finger der rechten Hand breit perforierte. Kubo (1912) hat schon damals bewiesen, daß eine in die Pars membranacea gesetzte große Öffnung die Neigung hat dauernd weit zu bleiben. Ich glaube, daß es heute bei den verfeinerten diagnostischen Methoden der Nasenendoskopie gepaart mit operativen Eingriffen unter Führung der Nasenoptiken absolut möglich ist, ausgewählte Fälle von rezidivierenden Kieferhöhlenempyemen durch Resektion der Fontanelle zur Heilung zu bringen. Grundbedingung dafür ist allerdings, daß die Kieferhöhlenmucosa keine irreversiblen Ver

änderungen zeigt. Das in der Fontanelle neu angelegte Ostium bleibt weit, wie schon Kubo (1912) nachwies und wie ich bei eigenen Fällen beobachten konnte. Auch der Sekrettransport durch dieses neue Ostium geschieht nach Epithelisierung auf normale Weise, da das Epithel von den Wundrändern her nachrückt und damit die richtige Transportrichtung in den mittleren Nasengang beibehält. Daß operativ angelegte Kieferhöhlenostien voll funktionstüchtig werden können, konnte ich schon vor Jahren in dem Film „Sekrettransport und Flimmerbewegung in den Nebenhöhlen des Menschen" zeigen.

RÉSUMÉ

La fontanelle du sinus maxillaire, d'un intérêt purement académique jusqu'à maintenant, peut être observée entièrement grâce à l'endoscopie nasale. Notre contribution porte sur son extension, sa structure et sa fonction. Dans le secteur des fontanelles certaines maladies peuvent entraîner des altérations qui, dans le cas d'une inflammation aiguë, varient entre une protrusion pulsative et la formation d'un abcès, dans le cas d'une sinusite chronique entre une protrusion, une formation de polypes solitaires, une concrétion dans une valvule et un cholestéatome. Des tumeurs malignes peuvent également avoir leur origine dans une fontanelle. Il arrive aussi que, à la suite d'une atrésie choanale, des trabécules osseux apparaissent dans le secteur postérieur des fontanelles.

SUMMARY

After a short description of the anatomy histology and the possible function of the nose fontanellae, their various changes already known from the literature are quoted. By means of nasal endoscopy the fontanellae can be seen very well. In acute sinusitis maxillaris they protrude by inflammation and pulsate in toto and fill their space in the middle nasal duct. In chronic inflammation they are vaulted, wedgelike or nipple-shaped, often with pigmentation in the mucosa. There can be formation of abscesses or concrements within a pouch or protrusion of a "choanal polypus" out of an accessorious ostium. In chronic rhinitis by the way of hyperplasia of the nasal part of the mucosa, the fontanella looks rim-like or wedged. Metaplasia of the epithelium may produce in the middle fontanella a cholesteatoma of the maxillary sinus. Also, malignant tumours may originate from the pars membranacea. Finally we have considered whether by means of nasal endoscopy it might be possible to cure various diseases by endonasal procedures, as has already been tried.

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Acta Otolaryng 73 296–303 1972

SILASTIC IMPLANTS IN THE EARS OF CATS

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Abstract. Silastic was examined in experiments on cats in order to determine its inertness in the ear and to assess its possible usefulness in middle ear and oval window surgery. The results indicated that properly prepared Silastic sheeting is inert in the mesotympanum and may have a useful part to play in tympanoplasty by promoting regeneration of mucosa and lessening of adhesions. Its usefulness in the confined spaces of the attic was less certain. Silastic sponge inserted after total stapedectomy provided inadequate protection of the internal ear and was associated with a high incidence of cochlear degeneration. Insertion of a Silastic piston after a stapes footplate fenestration was likewise associated with severe internal ear damage.

he silicones have found many uses in various nches of surgery because of their extreme ertness, although Silastic (Dow Corning Corp) is still the only medical grade silicone which is perhaps suitable for permanent implantation It was decided to examine Silastic by a series of operations on the ears of cats. Since this experiment began there have been a number of reports of Silastic being used in human middle ear surgery and many otologists now use this material, especially in tympanoplasty Regrettably there has been almost no experimental work reported from observations made on the ears of experimental animals.

Silastic was examined in three groups of experiments as follows: (*a*) in sheet form for experiments concerned with its tolerance in the middle ear and with tympanoplasty (*b*) as a fine sponge to close the oval window after total stapedectomy (*c*) as a rubber piston for use after fenestration of the stapes footplate

Silastic film in the middle ear and in tympanoplasty

In the first group of experiments Silastic sheeting was inserted into the normal middle ear into the ear freshly denuded of mucosa and into the ear being subjected to tympanoplasty

Silastic film was inserted into 4 normal ears, the animals were killed by intravital perfusion and fixation up to 30 weeks later and the temporal bones processed using conventional celloidin techniques. In each case the implant appeared to be entirely inert, no adverse effect was detected from its presence. In certain instances the mucosa had grown along the surface of the implant for a substantial distance (Fig. 1). This mucosa was of normal ciliated columnar type, although its submucosa was slightly thickened and of increased vascularity Further along the implant the epithelium was of reduced height and eventually of flattened type

Silastic film was next inserted into the mesotympanum of 5 ears from which the mucosa had been removed as completely as possible under microscopic vision immediately before insertion of the implant. When examined 16 weeks later the denuded areas were foun to be covered by mucosa that looked eithe normal or of fairly good quality Occasionally mucosa had grown onto the implant as alread seen, or had become slightly heaped u around the sharp end of the implant (Fig. 2) In one animal in this group the removal o

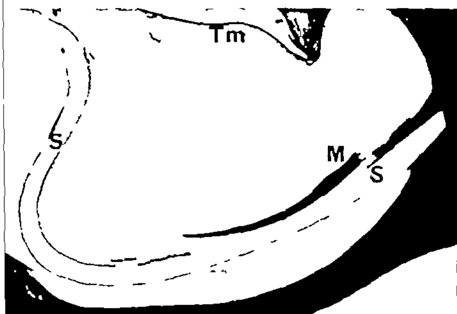


Fig. 1 Silastic sheet into normal mesotympanum. Middle ear mucosa (*M*) has grown for a substantial distance along the surface of the Silastic sheet (*SS*) which is entirely inert. Tympanic membrane is seen at (*Tm*). This and certain other illustrations are under exposed and over-printed in an attempt to illustrate the site of the Silastic sheet which almost invariably falls out of the histological sections and merely shows up as an empty space against a clear background (20)

mucosa was somewhat too vigorous and resulted in dislocation of the stapes. This complication was recognised during the operation and the Silastic implant accordingly was positioned over the promontory and oval window area in order to try to provide some protection of the internal (Fig 3) ear The internal ear was subsequently found to be normal in appearance on light microscopy apart from a minor degree of endolymphatic hydrops.

Following these preliminary experiments with Silastic in the normal ear and in the ear



Fig. 2. Silastic sheet into mesotympanum freshly denuded of mucosa. In this animal the Silastic sheet (*SS*) extended through most of the mesotympanum. At the point where it touches the mucosa the mucosa has become sl' ened. Tympanic (20).

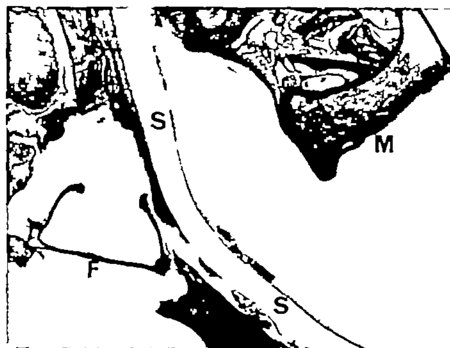


Fig. 3 Silastic sheet in the traumatised middle ear The deliberate efforts to produce as much mucosal removal and damage as possible caused dislocat of the stapes footplate (*F*) The Silastic sheet (*SS*) has closed off this area from the remaining parts of mesotympanum. The sheet lies in contact with normal-looking regenerated mucosa on the promontory The malleus is seen at (*M*) (×20).

newly denuded of mucosa, Silastic was evaluated in animals in which a two-stage operation was carried out. The first stage was designed to produce a situation comparable to adhesive deafness. The mucosa was removed as completely as possible the incus, head of malleus, or both ossicles were removed, and the ear allowed to fill with blood clot. Four animals were dealt with in this way and after 14 weeks the ears were re-opened. At this second-stage operation the tympanoplasty was carried out. Scar tissue was excised as completely as possible from the attic and mesotympanum the ossicles were re-inserted as autografts. Silastic sheets were inserted into the mesotympanum, the lateral and medial attic spaces around the ossicles, as well as over the atticotomy defect. The animals were sacrificed after a further 16 week interval. It was found that good quality mucosa had regenerated and that adhesions in the mesotympanum were minimal. Although the stapes had, unknowingly been subluxated either at the original operation or at tympanoplasty in one animal, only minor changes were present in the internal ear and these were considered probably to be the result of preparation artefact (Fig. 4)

Atticotomy in the cat is almost invariably followed by extensive prolapse of the overlying soft tissues into the attic spaces so that extensive scar tissue formation occurs within the attic. In these tympanoplasty experiments, therefore, Silastic sheeting was inserted around the ossicles in the attic as well as over the bony atticotomy defect. The Silastic film inserted over the atticotomy frequently became wrinkled or curled on itself and sometimes partly displaced, probably due to the action of the overlying temporalis muscle (Fig 5). Generally the implant became enclosed in a very delicate connective tissue envelope, although it was not unusual to see bone growing along one or both surfaces of the implant. In comparison with the controls there was a reduced tendency for the overlying soft tissues to prolapse inwards. Silastic sheets placed within the attic spaces to surround the ossicles, after removal of scar tissue at these tympanoplasty operations sometimes appeared to play a part in preventing further adhesion formation (Fig. 6) but more often implants in these



Fig. 4 Tympanoplasty The Silastic sheet (*SS*) is folded round the handle of the malleus. No adhesions are present between this and promontory although the mucosa (*Mm*) over the promontory is regenerated and somewhat thickened in appearance. The stapes footplate (*Fp*) has been accidentally subluxated but the internal ear was normal (20).

mall attic spaces seemed to be of doubtful alue and frequently became engulfed in new tissue without either promoting formation of new mucosa or of diminishing the severity adhesion formation. The underlying automalleus and/or incus were re modelling " in every case compared with controls hich no Silastic was inserted.

The results obtained from this first group of suggest that properly prepared lastic sheeting is highly inert in the normal m of the cat as well as in the which has been freshly denuded of mucosa. The mucous membrane has great powers of recovery in the mesotympanum and it would appear that Silastic, by ing the denuded surfaces physically sepa can prevent their adhesion together Silastic also appears to be highly inert in the of cats which are subjected to tympanoplasty and likewise may have a part to play in ing regeneration of mucosa in the mesotympanum and lessening the tendency to adhesion formation In the attic implants are likewise very inert, they may sometimes help to diminish adhesion formation around the ossicles, but the advantage of implants in confined spaces such as the attic could not be confirmed in these experiments. Silastic in no way interferes with normal remodelling processes of autograft ossicles. The work of Stengl & Hohmann (1964) and of Wilson et al. (1966, 1970) has now shown that homograft ossicles in cat and in man remodel in the same way as autografts. It is reasonable to suppose therefore that homograft ossicles are similarly unaffected by Silastic implants.

Total stapedectomy: Silastic sponge implant

In the second group of experiments total stapedectomy was carried out on 8 ears and a piece of Silastic sponge carefully fitted into the oval window in order to assess the suitability of Silastic sponge as a stapes footplate prosthesis and to enable comparison to be made with soft tissue autografts. The maximum survival time allowed was 56 weeks.

Only in one ear did the implant remain in situ in the oval window (Fig. 7). In this ear the implant was excessively bulky and consequently was somewhat wedged into position. It became surrounded by a delicate connective

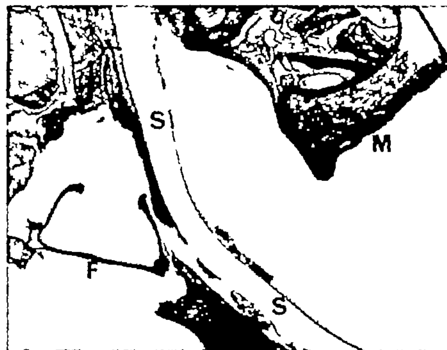


Fig. 3 Silastic sheet in the traumatised middle ear The deliberate efforts to produce as much mucosal removal and damage as possible caused dislocation of the stapes footplate (*F*). The Silastic sheet (*SS*) has closed off this area from the remaining parts of mesotympanum. The sheet lies in contact with normal-looking regenerated mucosa on the promontory. The malleus is seen at (*M*) (×20).

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Fig. 7 Oval window sponge implant. This was the only animal in which the sponge (*S*) remained *in situ*. It is excessively bulky. It is contained in an extremely delicate connective tissue envelope which on the middle ear surface is covered by mucosa (*A*) and on the internal ear surface by endosteum (*B*). The saccule is compressed onto its macula (*M*) (×18).

ably had formed fairly early In one of these 3 ears the Silastic sponge had displaced inwards into the vestibule A minor degree of endolymphatic hydrops was present, but otherwise no abnormalities could be detected on light microscopy

In one ear in the group a lesion resembling reparative granuloma was present and was associated with diffuse internal ear damage. Otitis media occurred in one animal and had led to a diffuse labyrinthitis.

The results in this second group of experiments indicate clearly that Silastic sponge is totally unacceptable as an oval window implant after stapedectomy Although the material appears to be inert it displays no tendency to adhere to the oval window edges and consequently becomes displaced easily Even if the sponge were to be maintained *in situ* as part of a prosthesis clamped onto the incus it still remains doubtful whether adequate cochlear protection would be obtained. The results of these experiments with Silastic sponge were totally inferior in every way to the results previously obtained with various tissue autografts such as connective tissue and fat. Moreover it appears that the complication of reparative granuloma is not one that is confined to soft tissue autografts. It is interesting to note that somewhat disappointing results have recently been reported by Paparella & Sugiura (1968) after using Silastic sponge in human stapedectomy It is now clear from these animal experiments that the risks are totally unacceptable when translated into human terms.

Stapes footplate fenestration. Silastic piston insert

In the final group of experiments fenestration of the stapes footplate was carried out and a piston prosthesis fashioned from Silastic rubber was inserted. The survival time for these animals was 26 weeks. In the 3 ears in this group severe and diffuse internal ear damage was present. Although the piston was in good position there was never any attempt to produce either a layer of mucosa over it or a layer of endosteum beneath (Fig 8) Lymphocytes and histiocytes were sometimes present in the oval window area around the implant and were associated with a serofibrinous type of reaction in the adjacent part of the vestibule. Otitis media in one ear was associated with total disruption of the internal ear and calcification in the cochlea.

The results of experiments in this third group of animals were even more unsatis-



Fig 8 Footplate piston. The piston (*P*) has re mained in satisfactory position. Some debris is present between the stapes crura. There was severe internal ear damage with total hair cell loss in the cochlea (*A*) (×20).

factory than those seen with the sponge implants. It would appear that all the disadvantages associated with Silastic sponge implants apply to operations in which Silastic pistons were examined, the only difference being that the risks were even greater

The overall results of the three groups of experiments suggest that

(i) Silastic sheeting is inert in the meso- um and may have a useful part to play tympanoplasty by promoting regeneration of mucosa and lessening of adhesions. In confined spaces such as the attic, however no convincing benefit could be demonstrated

(ii) Silastic sponge in the oval window fails to provide adequate protection of the internal ear after total stapedectomy

(iii) Insertion of a Silastic piston after stapes footplate fenestration in the cat merely seems to maintain patency of a channel into the vestibule which is associated with severe damage in the internal ear

ZUSAMMENFASSUNG

Silastic wurde in Experimenten an Katzen untersucht, um seine Reaktionslosigkeit im Ohr und seine mögliche Gebrauchsfähigkeit im Mittelohr und der Fenestra Ovalis Chirurgie festzustellen. Die Resultate lassen darauf schliessen, dass richtig vorbereiteter Silasticfilm im Mesotympanum reaktionslos ist und daher eine nützliche Rolle in der Tympanoplastik durch die Anregung der Schleimhautbildung und der Verringerung von Verwachsungen spielen dürften. Seine Gebrauchsfähigkeit im begrenzten Bereich des Epitympanum war weniger sicher Silasticschwamm, eingesetzt nach totaler Stapedektomie hat unzulänglichen Schutz des inneren Ohres geboten und war mit einer hohen Zahl von cochlearen Degenerationen verbunden. Einpflanzung von einer Silastic Piston nach einer Stapesfussplattenfenestration war gleichermassen mit schwerem Innerohrschaden verbunden.

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DISCUSSION

I Padovan. The experimental work of Mr Colman is very interesting and deserves special attention, espec-

ally nowadays when we use the alloplastic materials more and more often for different purposes. We have experience with Silastic sponge and we use this material very often in plastic and cosmetic interventions in the face especially in mentoplasty or in restoration of the forehead deformalities after frontoethmoid injuries. Morris Parker John Conley and the others have special experience with this material. According to my personal experience the Silastic sponge material is very well accepted by the human body especially subcutaneously. We have tried to use the Silastic sponge also submucously in cases of ozaena, i.e. between the mucous membrane and the bone of the nasal floor and between the perichondrium of the nasal septum and cartilage. In these cases the reaction in the nose is severe in the first 4 weeks following the operation and in the sixth week after the operation the elimination of the Silastic sponge starts so that at the end of the third month after operation the whole alloplastic material is expelled from the mentioned spaces. My question is: has Mr Colman any explanation why the Silastic sponge is well accepted in subcutaneous applications and why it is not accepted in the submucosa?

M Portmann. Je voudrais verser au dossier du Silastic mon expérience. J'ai fait une série de 104 malades à titre expérimental avec membrane de Silastic dans des tympanoplasties. Mes résultats ont *été mauvais — 30% de bons résultats seulement. Une* autre série de 8 cas a été faite avec éponge de silicone dans l'oblitération de la mastoïde, les 8 cas ont été tous mauvais.

H L Wullstein The difference of reaction subcutaneously compared with sponge in the middle ear is in my opinion derives from the fact that under the skin there is enough healthy tissue to react and to grow through the sponge. This we lack in the middle ear cavity the soft tissue there—even uninfected—is too little in quantity to handle the problem and to form enough granulating and later connective tissue to grow through and to create a quite definite situation of equilibrium between the sponge and the tissue

K Sedláček. The investigations of Mr Colman are very important and confirm our experiences with silicone implants. We have used for several years a silicone tabby in tympanoplasty operations to renew the mucous membrane in the middle ear I remove it after several weeks or 2-3 months and so obtain a cavity covered by a new epithelial lining I never saw any irritation of the tissues. I never use the artificial material directly in the windows and the findings of Mr Colman have proved that this precaution was very correct My question is, whether Mr Colman has investigated Silastic polymerized at the normal temperature by adding catalysers This kind of polymerization enables one to achieve impressions well adapted to the shape of the middle ear cavity

B H Colman (Reply) to Mr *Padovan* I do not know why Silastic implants should be better tolerated in subcutaneous tissues than in submucous tissues. These materials are probably equally inert in both sites, chemically speaking, but it has to be emphasised that physical properties concerned with surface and with shape are also important. Clearly an implant with a sharp edge will more easily migrate and will move more easily through mucosa than through tough skin. Nearby movement of tissues, especially muscles, will almost certainly encourage creeping of the implant. In the case of Silastic sponge in the oval window the essential problem, I think, is lack of stickiness to the oval window margins compared to tissue homografts so that displacement probably occurs rapidly before mucosa has had a chance to overgrow the implant and thereby fix it in position.

To Mr *Portmann*. Although Silastic film as demonstrated here is well tolerated and permits regeneration of fairly good quality mucosa in the mesotympanum after tympanoplasty it seems least effective where the need is greatest, i.e. in the small recesses of the attic and posterior mesotympanum. In these confined spaces around the ossicles the implants mostly became engulfed in scar tissue. The problem of mastoid cavity obliteration using Silastic sponge was not investigated, but I think the type of failure Mr Portmann describes arises from the fact that although the sponge may become satisfactorily surrounded by tissue it does not actually become incorporated into them. Furthermore although Silastic in any form is chemically highly *inert it still demonstrates the properties of any foreign* body in respect of shape and other physical properties and probably like any other properly prepared and shaped inert material used for implantation the deeper it is implanted the more likely will it stay

To Mr *Wullstein* I think my reply to Professor Portmann has already covered certain points raised by Professor Wullstein. Silastic sponge is infiltrated by the surrounding tissues only at its most extreme periphery by very short finger-like ingrowths of connective tissue. In no instance in which the sponge remained in situ in the histological sections was there any attempt whatever for the host tissues to infiltrate into the interstices of the sponge, even after 56 weeks.

To Mr *Sedláček*: I share your anxiety about leaving these implants permanently in the ear In the case of one or two implants located over the atticotomy defects and in close relation with soft tissues there was sometimes a patchy collection of lymphocytes and histiocytes. Maybe these were caused by tissue necrosis or by leakage of contaminant from the implant (most unlikely) or by improper preparation, cleaning and handling techniques on my part. Whatever the cause I suspect there are now similar reactions occurring in many human ears. You also referred to the use of cold cure polymers which can be introduced in fluid form and hardened in situ so that they correspond exactly in size and shape to the cavity which needs to be obliterated. I believe one of the dangers with these materials is that polymerisation may be incomplete. Delayed leakage of the vulcanising agent, or of incompletely polymerised material can cause severe tissue reactions.

Acta Otolaryng 73 305–315 1972

SALIVARY GLAND ADENOMAS OF THE PALATE

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(Received June 10, 1971)

Abstract. A histological survey of 383 tumours primary in the palate revealed a salivary gland tumour in 170 cases; 95 benign and 75 malignant. The benign tumours were all adenomas, 61 of pleomorphic and 34 of monomorphic type. Three of the 75 malignant cases were denoted as carcinomas in pleomorphic adenoma. The incidence of monomorphic adenomas is higher in the palate than in the parotid and submandibular glands. Lack of encapsulation was found a common feature but was not considered a histological sign of malignancy. In 88 patients with adenomas followed up for 5–45 years, local recurrences arose in 7 cases, in 5 with pleomorphic and in [illegible] with monomorphic adenoma. None of the patients with a salivary gland adenoma had metastases or died of the tumour disease. There was no difference in the clinical outcome between patients with pleomorphic and monomorphic adenomas. Two of the 3 patients with carcinoma in pleomorphic adenoma succumbed to metastasizing tumour and the third patient died of intercurrent disease. Pleomorphic and monomorphic adenomas of the palate were found to be benign tumours. A large number of the tumours, however lacked a well defined fibrous capsule, thus involving a risk of recurrence after surgical removal of the tumour. Because of this and since benign salivary gland adenomas in the palate could not clinically be distinguished from such with a malignant component (cancer in pleomorphic adenoma) the importance of primary radical surgery and careful histological examination of the operation specimen is stressed.

Adenomas of various kinds are by far the most common type of tumour originating from the salivary glands. Two main types of adenoma occur: pleomorphic (mixed tumour) and monomorphic. A pleomorphic adenoma is characterized by a mixture of epithelial and mesenchymal components. Monomorphic adenomas can be divided into three subgroups, adenolymphomas, oxyphilic adenomas (oncocytomas) and adenomas of other types. In monomorphic adenomas the histological structure is uniformly epithelial, with exception of adenolymphomas which in addition have a characteristic lymphoid component. Fibrous or small myxoid areas may occur among the epithelial structures of the monomorphic adenomas but should not be used to classify an otherwise monomorphic adenoma as pleomorphic. Adenolymphomas and oxyphilic adenomas have very characteristic histological features (Foote & Frazell, 1954, Hamperl, 1962, Kleinsasser 1969). These tumours are common in the parotid gland and occur in exceptional cases in the submandibular gland (Eneroth, 1964, Eneroth & Hjertman, 1967). No such tumours have, however been reported to originate from the minor salivary glands of the palate.

PRESENT SERIES

The present study is based on an analysis of histologically verified tumours of the palate in 383 patients registered at Radiumhemmet during the period 1909–1966. A histological re-examination was made of all the tumours, which were then reclassified according to a more differentiated nomenclature than the original one (Foote & Frazell, 1954, Eneroth, 1964). The prerequisites for a histological

survey exist, since the tumour material is available at the Department of Tumour Pathology With few exceptions all the patients have been re-examined regularly and it was therefore possible to carry out a histological-clinical correlation study

The histological re-examination of the palatal tumours in the 383 patients disclosed a salivary gland tumour in 170 cases, 95 of which were histologically benign and 75 malignant. The 95 histologically benign salivary gland tumours of the palate were all monomorphic or pleomorphic adenomas. None of the monomorphic adenomas had the histological characteristics of adenolymphoma or oxyphilic adenoma. Of the 75 malignant salivary gland tumours 37 cases were classified as adenoid cystic carcinoma (Eneroth et al., 1968) and 27 cases as muco-epidermoid carcinoma (Eneroth et al., 1970) Of the remaining 11 cases, 8 tumours were recognized as different types of adeno-carcinomas, 5 solid, poorly differentiated, 2 adenopapillary carcinomas and 1 mucus-producing adenocarcinoma. Three cases were considered as carcinomas in pleomorphic adenoma.

The present study concerns the 95 patients n pleomorphic and monomorphic adenoma the 3 patients with carcinoma in pleo- adenoma. These 98 patients were treated during the 1921–1966 period.

The clinical follow up study was based on 91 of these 98 patients treated in 1921–1961 i.e., with a minimum observation period of 5 years.

The prognosis of the tumours of the palate was investigated by studying the rate of local recurrence, metastasis, mortality and survival rate. The survival rates were based on determinate groups, from which were excluded the indeterminate patients, i.e those lost to follow up and those who died without signs of tumour disease. Only one patient in the present series was lost to follow up. A long observation period is necessary to obtain reliable prognostic figures. In the present series, the follow-up period ranged from 5–45 years.

HISTOLOGICAL FEATURES

Sixty-one of the benign adenomas of the palate were considered to be of the pleomorphic type and the remaining 34 cases of the monomorphic type.

Pleomorphic adenoma

The pleomorphic adenomas (mixed tumours) exhibited the same histological features as the corresponding tumours developing in the major salivary glands (e g. Eneroth, 1964). The tumours were characterized by epithelial and mesenchymal components in varying proportions. The epithelial cells formed tubular alveolar and solid structures and exhibited no cellular polymorphism. Mitotic figures were rarely seen. In 5 cases the epithelial component showed squamous metaplasia with a tendency to keratinization (Fig. 1) The mesenchymal component was mostly fibroid or myxoid, in a few cases with chondroid areas. A great number of the tumours had no well marked fibrous capsule.

Monomorphic adenoma

The monomorphic adenomas were distinguished by a dominance of a cellular epithelial component with only a scant supportive fibrous stroma. Single, minute areas with myxoid differentiation were accepted without registering the tumour as a pleomorphic adenoma. The epithelial component in the tumours was either tubular/alveolar 17 cases (Fig. 2) solid/trabecular 15 cases (Fig. 3), or of the clear cell type 2 cases (Fig. 4). None of the tumours showed squamous metaplasia. A few of the solid, monomorphic adenomas showed some cellular polymorphism but in no case was this so marked that malignancy was suspected. The pleomorphic as well as the monomorphic adenomas in the palate mostly lacked a well defined fibrous capsule and diffuse tumour growth into the adjacent tissues could simulate true invasive growth (Fig. 5). The absence of cellular polymorphism and

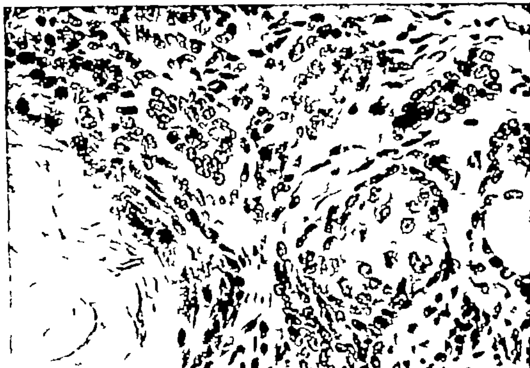


Fig 1 Pleomorphic adenoma. Squamous metaplasia of the epithelial component. Tendency to keratinization. Photomicrograph, 400.

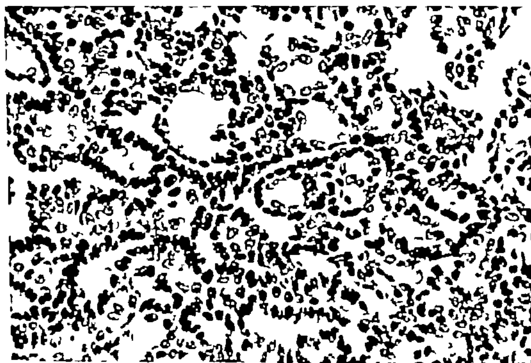


Fig 2 Monomorphic adenoma. Well differentiated epithelial component with tubular structures. Photomicrograph, 400.

Fig. 3. Monomorphic adenoma. Solid epithelial component with little cellular polymorphism. Scanty fibrous stroma. Photomicrograph, × 150.

Fig. 4. Monomorphic adenoma. Epithelial cords composed of "clear" cell types. Photomicrograph, × 400.

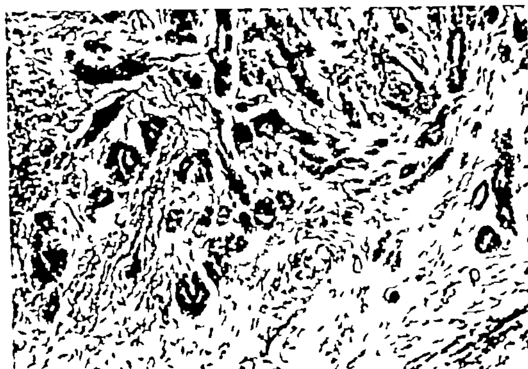


Fig 5 Monomorphic adenoma. Diffuse growth without capsule formation into adjacent tissue. Photomicrograph, 150.



Fig 6 Carcinoma in pleomorphic adenoma. Anaplastic carcinomatous component resembling a fibrosarcoma. Photomicrograph, 400.

few mitotic figures revealed the benign nature of the lesion in such cases.

Carcinoma in pleomorphic adenoma

Three cases were diagnosed as cancer in pleomorphic adenoma. All tumours appeared as truly pleomorphic. In one case the malignant component appeared in the form of large cell anaplastic carcinoma resembling the granular cell proliferation found in acinic cell carcinomas. In the second case the malignant growth was a partly solid, somewhat polymorphous adeno-carcinoma. In the third case the malignant component appeared as a diffusely growing anaplastic carcinoma which showed such resemblance with a fibrosarcoma that it primarily was diagnosed as such (Fig. 6)

CLINICAL FEATURES

Pleomorphic adenoma

Of the 61 patients with pleomorphic adenoma of the palate, 30 were men and 31 were women. At the time of histological diagnosis the age of the patients ranged from 13–77 years and the mean age was 46.5 years. The duration of the symptoms was less than 1 year 35 cases, between 1 and 5 years in 16 cases , more than 5 years in 10 cases. The mean of the symptoms was 2.6 years. Forty-eight of the 61 patients with a pleomorphic adenoma of the palate observed a lump in the palate and in 34 patients this lump was the only symptom of the tumour disease. Other symptoms were pain (6 cases) bleeding (4 cases) and denture troubles (9 cases). Blocked nasal passage and difficulty in swallowing appeared in 4 patients, each of whom had a tumour more than 4 cm in diameter In 5 cases the tumour was detected incidentally In 37 cases the tumour was localized to the hard palate, in 21 cases to the soft pelate and in 3 cases to both the hard and soft palate. In 13 cases the diameter of the tumour was less than 2 cm, in 30 cases between 2 and 4 cm and in 18 cases more than 4 cm. The surface of the tumour was ulcerated in 6 cases, all of which were localized to the hard palate. In 2 cases it was noted that the ulceration was caused by a prosthesis.

Monomorphic adenoma

A monomorphic adenoma appeared in 34 patients, 13 men and 21 women. The age of the patients at the time of histological diagnosis of the tumour and treatment ranged from 15 to 75 years (mean age 50 1) The interval between the appearance of the first symptoms of the tumour and its treatment ranged from a few days to 27 years. In 21 cases the duration of the symptoms was less than 1 year in 5 cases between 1 and 5 yeras and in 8 cases more than 5 years. The mean duration of the symptoms was 4 0 years. The commonest symptom of the tumour disease, a lump in the palate, was present in totally 28 cases and as the only symptom in 19 cases. Other symptoms were pain (5 cases) bleeding (3 cases) and denture difficulties (5 cases) In 4 cases the patients were completely free from symptoms, when the tumour was detected incidentally In 23 cases the tumour was localized to the hard palate, in 8 cases to the soft palate and in 3 cases to both the hard and soft palate. The diameter of the tumour was less than 2 cm in 7 cases, between 2 and 4 cm in 20 cases and more than 4 cm in 7 cases. In 3 cases the mucous membrane over the tumour was ulcerated and in 2 of these cases the diameter of the tumour was more than 4 cm. The 3 tumours with ulcerated surface were situated in the hard palate.

Carcinoma in plemoorphic adenoma

The 3 patients with carcinoma in pleomorphic adenoma were men 43 51 and 64 years old respectively the mean age being 52.7 years. The interval between appearance of the first symptoms of the tumour and its treatment was in 2 cases very long, 10 and 15 years, in the third case, however only 2 years. A lump in the palate was the only symptom of the tumour in 2 of the patients. One patient complained of pains in the neck and ear In 2 cases the

tumour was localized to the hard palate and in 1 case to the soft palate. The diameter of the 3 tumours was more than 4 cm. The tumour in the soft palate was diffusely circumscribed and had extended to the lateral pharyngeal wall. The mucous membrane over the tumour was not ulcerated in any of the 3 cases.

TREATMENT

Pleomorphic and monomorphic adenoma

The treatment of the pleomorphic and monomorphic adenomas of the palate was surgery irradiation or a combination of the two forms of therapy All patients were operated upon, but in 2 cases the surgical intervention was small and is rather to be denoted as a biopsy Excision of the tumour was done in totally 84 cases, in combination with electrocoagulation in 13 cases and in combination with resection of the bony palate and neck dissection in 1 case. In 9 cases the tumour was electrocoagulated. Irradiation was given in 68 of the 93 operated cases and in the 2 cases in which the surgical procedure is denoted as only a biopsy

In 56 of the 70 irradiated patients the tumour was denoted as semimalignant or malignant according to the existing nomenclature at the time of treatment. The 2 patients who after biopsy of the tumour were only irradiated, were treated before 1940, while 21 of the 25 patients, who were only operated upon, were treated in 1950 or later

Carcinoma in pleomorphic adenoma

The treatment of 3 cases of carcinoma in pleomorphic adenoma was irradiation, in 1 case in combination with excision and electrocoagulation of the tumour In 2 cases the surgical intervention was small and can be denoted as only a biopsy

PROGNOSIS

Recurrence

Altogether 88 of the 95 patients with a pleomorphic and monomorphic adenoma of the palate were treated during the 1921–1961 period and could thus be followed up for more than 5 years. During the follow-up period a local recurrence occurred in 7 patients, 5 with a pleomorphic adenoma and 2 with a monomorphic adenoma. Six of the 7 patients with a recurrent tumour were treated in 1945 or earlier

The recurrences occurred in 5 cases within 5 years and in 2 cases after 13 and 14 years respectively In 6 cases the recurrences were excised, in 1 of these 6 cases in combination with irradiation, while the recurrence was not treated in 1 case—an 84-year-old patient. The average observation time of the 6 patients after treatment of the recurrences was 18.5 years and during this follow-up period occurred 1 new recurrence after 7 years. The recurrent tumour was excised and the patient was then free from further recurrences during a new observation time of 24 years.

The 3 patients with a carcinoma in pleomorphic adenoma were followed up for more than 5 years. In 2 cases no local recurrence occurred, whereas in the third case a local recurrence occurred 6 years after the first treatment.

Metastases

Neither regional lymph node metastases nor distant metastases were observed in any case of pleomorphic or monomorphic adenoma of the palate.

Regional lymph node metastases were observed in 1 case and distant metastases in another case of carcinoma in pleomorphic adenoma. The lymph node metastases occurred in the submandibular region and the distant metastases in the skeleton.

Survival

None of the 88 patients with pleomorphic and monomorphic adenoma died in the tumour disease during the follow-up period of 5–45 years. Eighty-eight patients had been under observation for at least 5 years, 76 for at least 10 years, 66 for at least 15 years and 52 for at least 20 years. Two patients treated in

the 1930s, however died in complications to the surgical procedure One patient was lost to follow-up after 10 years.

Two patients with carcinoma in pleomorphic adenoma died of the tumour disease 1 and 6 years, respectively after treatment. One of these 2 patients succumbed to local recurrence and regional lymph node metastases and the other to metastases to the skeleton. The third patient with carcinoma in pleomorphic adenoma survived the tumour disease for 9 years but then died of intercurrent disease

DISCUSSION

Pleomorphic adenomas constitute the majority of adenomatous tumours arising in the salivary glands (Eneroth, 1964 Eneroth & Hjertman, 1967 Dahlin 1968). These tumours are characterized by a mixture of epithelial and mesenchymal components in varying proportions. Frequently however one component dominates the tumour structure Such tumours which appear entirely epithelial are recognized as monomorphic adenomas.

In the present series 61 tumours were classed as pleomorphic and 34 cases (35%) as monomorphic adenomas. The relative number of monomorphic adenomas among salivary gland tumours occurring in the palate was thus high compared with the corresponding figures for tumours arising in the major salivary glands (e.g Eneroth & Hjertman, 1967) In another 3 cases of pleomorphic adenoma the tumours exhibited a carcinomatous component and were hence classified as carcinoma in pleomorphic adenoma.

The 61 pleomorphic adenomas of the palate in the present study are equally distributed between men and women, while more than 60% of the 34 monomorphic adenomas occurred in women. The sex distribution of the pleomorphic adenomas varies in different tumour series. Thus according to Chaudhry et al. (1961), there is a female preponderance of pleomorphic adenomas in the minor intraoral salivary glands, while the sex distribution was equal of pleomorphic adenomas of the palate according to Dahlin (1968) Pleomorphic adenomas of the submandibular gland were nearly twice as common in women as in men (Simons et al. 1964 Eneroth & Hjertman, 1967).

Pleomorphic as well as monomorphic adenomas occur in all ages from the teens up to the old age. As regards the age distribution the figures in the present study are in agreement with those reported in the literature (Eneroth & Hjertman 1967 Dahlin 1968). The duration of the symptoms varied considerably some patients immediately applied to a doctor when feeling discomfort in the palate while others have had their troubles for up to more than 20 years. As a rule, however the duration of the symptoms was very short, in 58% of the cases 1 year or shorter The commonest sign of the tumour was a rounded lump No less than 80% of the patients had this symptom, whereas the more dramatic symptoms such as pain and bleeding were rare. Every sixth patient had difficulty in wearing dentures and every tenth patient was free from symptoms when the tumour by chance was detected at inspection of the oral cavity This fact illustrates the importance of the dentist in detecting tumours of the palate. The tumours were localized to the hard palate in two-thirds of the cases and in one-third to the soft palate. Similar observations were reported by Martin (1942) and Dahlin (1968) As a rule the tumours gave small symptoms in spite of a remarkable size in many cases in 31 cases the diameter was 4 cm or more The surface of the tumour was ulcerated in 9 cases (9.5%), which is remarkable in a histologically benign tumour All ulcerated tumours, however were localized to the hard palate some of them of very large size and in some cases it was noted that the ulceration was caused by the prosthesis. In malignant salivary gland tumours the frequency of ulceration is much higher 20% (Hjertman & Eneroth, 1970).

In the three cases of carcinoma in pleomorphic adenoma the mean duration of the

symptoms was lengthy and the tumours were large but in many cases of pleomorphic and monomorphic adenoma the same characteristics were present. From the clinical features only it was impossible to distinguish carcinoma in pleomorphic adenoma from pleomorphic and monomorphic adenoma.

The treatment of patients with pleomorphic and monomorphic adenomas of the palate varied during the long period the present material comprises. Excision of the tumour was the most common surgical intervention and was done in 88% of the cases. Irradiation was given in 74% of the total number of cases. Of the irradiated cases four-fifths were classified as malignant or semimalignant tumours according to the nomenclature valid at the time of treatment. The principles of the treatment has changed during the course of the period. Thus for instance before 1950 only 7% of the patients were treated exclusively with surgery. After this time, however surgery was the only treatment in 55% of the cases.

In the major salivary glands pleomorphic adenomas have a great tendency to recur when the treatment is merely excision of the tumour (Schreiner 1959 Grage et al., 1961 Beaven, 1963). A pleomorphic adenoma, which has once recurred, is much more likely to produce later recurrences (Eneroth & Hjertman, 1967). According to Chaudhry et al. (1961) recurrence developed in one out of 40 pleomorphic adenomas of the minor intraoral salivary glands and according to Dahlin (1968) recurrence developed in two out of 33 pleomorphic adenomas of the palate. Compared with the major salivary glands, however considerably less is known about the behaviour of adenomas in the minor salivary glands.

For the estimate of the frequency of recurrence of adenomas of the salivary glands a very long observation period is necessary (McFarland, 1933 Mylius, 1953 Eneroth & Hjertman, 1966). In the present material the observation period is very long, 5–45 years in 88 patients with pleomorphic and monomorphic adenoma. In these 88 patients there were 7 recurrences, one of them after as long a time as 14 years. The recurrences were equally distributed between pleomorphic and monomorphic adenomas in relation to the number of the two tumour types. The treatment of the recurrences was successful, only one new recurrence arose during a further observation time of 18.5 years.

The frequency of local recurrence of adenoma of the palate was surprisingly low considering the lack of a well defined fibrous capsule in numerous cases. Diffuse growth of the non-malignant adenomatous tumour into adjacent tissues seems to be a much more common feature of tumours in the palate than of corresponding tumours developing in the major salivary glands. Since a large number of the tumours in the palate were treated by means of minor surgery (local excision with minimal margin) before 1945 it is obvious that the absence of a capsule cannot be interpreted as a sign of malignancy of these tumours. It can also be emphasized that the generally accepted histological evidence of malignancy invasive growth, is inaccurate and hard to evaluate especially in salivary gland tumours of the palate. It is of interest that muco-epidermoid carcinomas of the palate frequently lack an obvious capsule. In spite of this local recurrences are rare after surgical removal of the tumour (Eneroth et al., 1970).

No patient with pleomorphic and monomorphic adenoma of the palate in this tumour series had regional lymph node metastases or distant metastases. Except 2 patients who died of complications to the surgical intervention during the 1930s, no patient died of the tumour disease.

Thus, the present study shows that pleomorphic and monomorphic adenomas of the palate are benign tumours. No metastases occurred and no patients died of the tumour disease during the very long follow-up period, 5–45 years. Adenomas have, however a tendency to recur but a radical primary surgical intervention reduces this risk to a very low level. No patient primarily treated at Karo-

linska Sjukhuset since 1945 has had any recurrence of an adenoma of the palate.

High cellularity has been regarded as a criterion of "semimalignancy" of mixed tumours of the salivary glands (Ahlbom 1935) McFarland (1942) found a higher rate of recurrence in the predominantly epithelial tumours than in the predominantly mesenchymal ones. In the present study however the pleomorphic and monomorphic adenomas of the palate are equal as regards recurrences and prognosis.

The true malignancy of the 3 cases considered as carcinomas in pleomorphic adenoma was confirmed by the clinical outcome. Two of the 3 patients died from metastasis, 1 and 6 years respectively after the initial treatment. The third patient died of an intercurrent disease In none of the 3 cases could the primary tumour be distinguished clinically from a benign tumour The malignancy was revealed by the histological examination. Because of this, and because a malignant component may occur in a small area within an otherwise benign tumour (Moberger & Eneroth, 1968) the study has stressed the importance of pri[illegible] radical surgery of adenomatous palatal [illegible] as well as the necessity of a careful [illegible]ogical examination of the tumour speci[illegible]

ZUSAMMENFASSUNG

Eine histologische Untersuchung von 383 Primärtumoren im Gaumen zeigte in 170 Fällen Speicheldrüsentumoren, von denen 95 benigne und 75 maligne Fälle waren. Die benignen Tumoren waren sämtlich Adenome, und zwar 61 von pleomorphem und 34 von monomorphem Typ. Drei der malignen Fälle wurden als Karzinome in pleomorphem Adenom klassifiziert. Monomorphe Adenome treten häufiger im Gaumen als in den Parotis- und Submandibularisdrüsen auf. Das Fehlen von Einkapselungen wurde als durchgehendes Merkmal konstatiert, jedoch nicht als histologisches Zeichen von Malignität betrachtet.

Bei 88 Patienten, die während einer Zeit von 5 bis zu 45 Jahren weiter verfolgt wurden, traten in 7 Fällen lokale Rezidive auf in 5 Fällen mit pleomorphem und in 2 Fällen mit monomorphem Adenom. Keiner der Patienten mit Speicheldrüsenadenom wies Metastasen auf oder starb an der Tumorkrankheit. In dem klinischen Resultat bestand kein Unterschied zwischen Patienten mit pleomorphem bzw monomorphem Adenom.

Zwei der 3 Patienten mit Karzinom in pleomorphem Adenom starben an metastasiertem Tumor Der dritte Patient starb an einer interkurrenten Krankheit.

Es wurde festgestellt, dass pleomorphe und monomorphe Adenome im Gaumen benigne Tumoren waren. Eine grosse Zahl der Tumoren besass jedoch keine feststellbare fibröse Kapsel, weshalb nach chirurgischer Entfernung des Tumors die Gefahr eines Rezidivs besteht. Auf Grund dessen und da benigne Speicheldrüsenadenome im Gaumen klinisch nicht von Adenomen mit maligner Komponente (Cancer in pleomorphem Adenom) unterschieden werden konnten, wird die Bedeutung primärer radikaler Chirurgie und einer gründlichen histologischen Untersuchung der Operationspezimina besonders hervorgehoben.

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Acta Otolaryng 73 316–322, 1972

SURFACE VIEW OF THE FROG VESTIBULAR ORGAN WITH THE SCANNING ELECTRON MICROSCOPE

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(Received February 2, 1971)

Abstract. The characteristic surface structure of the epithelial layer of the statoconia the otolithic membrane the macula statistica and the semicircular ampulla in frog was studied with scanning electron microscope paying particular attention to the structure of the otolithic membrane, the relationship of the reticular structure and sensory hairs, the form of sensory hair and the arrangement of sensory hairs in the sensory region of the semicircular ampulla, the detailed structure of which have not successfully been observed by electron or light microscopic methods. Further the free surface of the transitional zone the planum semilunatum and the gelatinous portion of the ampulla was demonstrated. New findings on structure of the gelatinous portion are also de

Light and electron microscopic studies have brought us many findings on the structure of the cochlear and the vestibular labyrinth. They have been limited, however chiefly to the cellular elements while the structure of the otolithic membrane crista ampullaris and semicircular canals has not yet been clarified, because of the unsuitability of conventional methods for the study of surface and solid structure. Recently Lim (1969), reported a three-dimensional observation of the surface of Corti's apparatus and the vestibular receptor and Kellerhals et al. (1970) and Rosenhall (1970) also reported the surface view of the otolithic membrane. Engström & Engström (1970) and Marovitz et al. (1970) also caught the fine structure of the hair cell region of Corti's apparatus with the scanning electron microscope (SEM). Thus the surface structures of these organs are gradually becoming clear However as to the morphology of the otolithic membrane and the ampulla, many important points remain unclarified because they lie deep in the bone and are very small and fragile.

In this study the author reports some new information on the surface structure of the ampulla, otolithic membrane, macula utriculi and macula sacculi of frog.

MATERIAL AND METHODS

The head of frog (*Rana nigromaculata*) was cut off the ampulla, macula utriculi and sacculi were excised in 2% glutaraldehyde buffered with Millonig phosphate solution after 4 hours fixation with glutaraldehyde 2% osmium for 1 hour and then another fixation with graded alcoholic dehydration was followed by acetone anhydride dehydration. For observation of the macula utriculi and sacculi, statoconia were dissolved with 10% HCl prior to dehydration. The warm air dried specimens were mounted on copper holders and coated with carbon and gold by vacuum vaporization on a rotating stage observation was made with a JSM U3 scanning electron microscope. For X ray analysis of the otolith a primary X ray analyser was used for the identification of calcium.

FINDINGS

Statoconia

The three-dimensional architecture of the statoconia could be observed more clearly and more precisely with SEM than by conventional light and electron microscopy. Statoconia showed crystallity of wide variation in size. The end of the crystal was pointed at approximately 90° angle forming three facets. These facet surfaces were smoother than the main crystal body (Fig. 1). The surface of the body appeared to be granulated, especially at high magnification (Fig. 2) and very small statoconia were seen attaching to the body of a large statoconia. In an analysis of its composition with a primary X-ray analyser which showed it to be a $CaCO_3$ crystal, the statoconia appeared on the Braun tube as a Ca X-ray image (Fig. 3).

Saccule and utricle

(a) *Structure of the saccule otolithic membrane.* The otolithic membrane has been studied by slice preparation, which failed to show its surface architecture in three dimensions.

By excision and dissection of the central part of the otolithic membrane the sensory hairs could be observed as a bundle of sensory cilia. The otolithic membrane showed a reticular architecture, into which the sensory hairs protruded (Figs. 4, 5).

In Fig. 4 the number of sensory hairs was calculated to be approximately 900. The membrane was fairly thick and covered only the sensory region.

(b) *The otolithic membrane of utricle and sensory hairs.* Observation of the surface architecture of the otolithic membrane of the utricle revealed that the meshes took rather round form as if they were punched out, while in the otolithic membrane of the saccule the membrane showed a chain-like appearance. However, it might be possible that this difference came from different solubility of the gelatinous substance on acid chloride treatment (Fig. 6). Fig. 7 provides evidence that the tip of the sensory hair protrudes into the otolithic membrane having a reticular architecture, and a bundle of sensory cilia can be observed within a round mesh.

In the central part of an approximately hexagonal sensory cell some thirty stereocilia were observed in 6–7 rows, each containing 4–5 stereocilia (Figs. 8, 9) thus forming "church pipe organ arrangement" (Lim, 1969).

In the part which corresponds to the extramacular portion near the sensory area in the saccule, hexagonal cells were seen as if benzol nuclei were linked (Fig. 10). On each cell one small grain-like point was observed, the nature of which remains still unknown.

The ampulla of semicircular canal

(a) *Sensory area of the crista ampullaris.* On the free surface of the crista, sensory cells were observed arranged in longitudinal rows. In a row longitudinal to the crista ampullaris 10–15 sensory cells stood in line, each with sensory hairs. But in the part near the planum semilunatum the number could not be counted in Fig. 11. The central part of this sensory cell showed (Fig. 12) a little elevation, in the centre of which a bundle of stereocilia was seen. There were 6–7 rows, each containing 4–6 stereocilia of approximately the same length. In each row the sensory cilia showed sequential length, thus exhibiting the "pipe organ arrangement".

(b) *Transitional zone.* The free surface of the part called transitional zone was, as seen in Figs. 13 and 14, somewhat raised above the level of the cell junction. It consisted of hexagonal cells of similar size, some of which showed granular rising on their surface.

(c) *Planum semilunatum.* The planum semilunatum is the part occupying both walls of the ampulla of crista ampullaris and is elevated in a semilunar form. This area makes a direct continuation of the sensory epithelium and





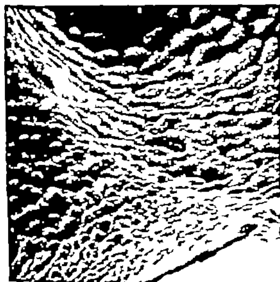


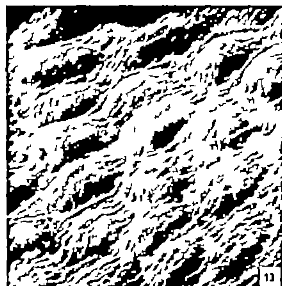


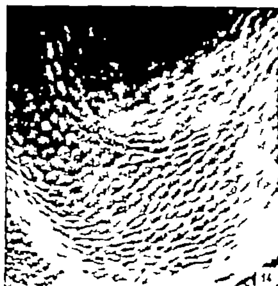
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the widest part of this area measures 400–500 μ in frog

(d) *Gelatinous portion* In frog there is a round elevated part having a diameter of approximately 300 μ and covered with gelatin ous substance at the bottom of both sides of ampulla divided through the crista am- ns. The author could not find any de- ption on this finding in the literature, and has named it "gelatinous portion" temporarily The surface of this portion seemed to be rather lower than the transitional zone possibly because of dissolution of the gelatinous substance on dehydration (Fig. 14).

DISCUSSION

A scanning electron microscope (SEM) which has been widely used in metallurgical investiga tions is now available for biomedical research. On Corti's organ, Lim (1969) and Engström & Engström (1970) have had some success on studying its surface architecture but on vestibular organs almost no detailed report has been given except for several photos by Lim (1969) Kellerhals et al. (1970) and Rosenhall (1970).

The author reports in this paper some of them for the first time more clarified findings of the surface architecture of statoconia, especially of otolithic membrane sensory hairs of the macula, the number and structure of sensory hairs, the number of cilia, the structure of the epithelium around the macula, and the surface architecture of sensory epithelium transitional zone planum semilunatum and the gelatinous portion of semicircular ampulla.

Statoconia

It is well known that statoconia consists of calcium carbonate. In statoconia there are aragonite and calcite, and Carlström et al. (1953) with the aid of X ray diffraction technique reported that the statoconia consisted of calcite in such mammals as homo, lepus and cavia, in birds such as columba and tetrao, and in shark while it consisted of aragonite in amphibia such as rana and bufo, and in bone fishes as perca, solea and salmo.

In light microscopic observation the frog statoconia shows various sizes and have the form of a Rugby ball. With the SEM it was observed that the cristal tip constituted of three facets, and exhibited a crystal with approximately 90 angle tip on the crystal body there appeared a fine granular structure

The angle of the tip of the crystal was reported to be 120 in homo lepus and cavia by Carlström & Engström (1955) and 109 in cavia by Kellerhals et al. (1970). The latter coincided with the figure reported by Iurato & DePetris (1967) in a calcite model tip

In frog the angle was somewhat smaller measuring approximately 90

Otolithic membrane and macula statistica

The otolithic membrane lies between the statoconia and the sensory hairs, by which the macula statistica can receive gravity and linear acceleration efficiently On this membrane a detailed study was made by Igarashi & Kanda (1969) who reported that on the sensory hairs lay the first subcupular zone, the second cupular zone 2, third cupular zone 1 and finally statoconia.

The author could not find any reports on a reticular architecture for this otolithic membrane, but Igarashi & Kanda (1969) and Kellerhals et al. (1970) reported that stereocilia and kinocilium protruded into the subcupular and cupular zones of the otolithic membrane and that the statoconia did not touch directly with sensory hairs. But Iurato & DePetris (1967) reported that the sensory hairs were in contact with statoconia in the macula statistica of rat.

In the present study sensory hairs protruded into the reticular architecture of the otolithic membrane. It might be the function of the otolithic membrane to let each sensory hair a bundle of sensory cilia, receive stimulation of gravity and linear acceleration accurately and also to put the sensory hairs into the reticular architecture to protect them.

Fig. 7 is one very valuable photo which established the fact that sensory hairs can be seen through the reticular architecture. Judging from this photo statoconia is not in direct contact with sensory hairs in frog. This reticular architecture might correspond to the subcupular zone in the report of Igarashi & Kanda (1969). The cupular zone might correspond to the gelatinous substance covering the surface of this reticular architecture.

In either case Figs. 4 5 6 and 7 seem excitingly interesting as the first to catch the surface of the otolithic membrane.

The macula statistica of the saccule were naturally larger than those of the utricle, but the form of the sensory hairs were the same and consisted of about 30–40 stereocilia and one kinocilium, the latter having been unidentified in the present work. The arrangement of stereocilia was described by Lim (1969) as "church pipe organ arrangement" They form 6–7 regular lines each containing 4–5 stereocilia. The number of sensory hairs of the saccule were computed to be some 900.

The author intends to examine whether the different surface morphology of otolithic membrane of the saccule and the utricle is due to a dissolution of the gelatinous substance by acid hydrochloride.

Semicircular ampulla

1) *Sensory epithelium of the ampulla.* Reports of SEM on the surface architecture of statoconia and macula statistica have been presented as mentioned above, while there have been no detailed reports on that of the crista ampullaris. Whilst the wall of the ampulla of mouse or guinea pig is very soft and so easily shrinks on drying in frog it is relatively strong enough to manipulate, though it is very difficult to dehydrate sensory hairs in their upright position, for the sensory cilia are long.

The sensory hairs of the crista ampullaris are regularly arranged. Therefore, if the relation between kinocilium and stereocilia is clarified in SEM, Flock s (1965) electron microscopic study will easily prove that the kinocilium exists on the side of the utricle in the lateral semicircular canal, whereas in the anterior and posterior semicircular canals it exists on the side of canal end Further the number of the sensory hairs over the surface of the ampulla can be directly calculated. Differences among various animals no-ticed. The sensory hairs of t ul

laris were far longer than those of the macula statistica.

2) *Transitional zone* The cells of the transition epithelium on both sides of the crista ampullaris was hexagonal and of approximately similar size. Watanuki et al. (1970) reported right hexagonal cells of various size in this part of guinea pig, on the surface of which they observed small granules.

3) *Planum semilunatum.* The planum semilunatum is the semilunar part elevated on both sides of the ampulla, named by Steifensand (1835) This part makes a direct continuation from sensory epithelium and fans out on both side walls of the ampulla, and further extends near the mid-line of the roof of the ampulla. It seemed that the sensory hairs are found in abundance in this part in frog, a more detailed examination of which will be reported at the next opportunity

Gelatinous portion. This was observed in frog, but not in guinea pig and mouse. It is the portion where the gelatinous substance lies over the free surface of the bottom of the ampulla on both sides of the crista ampullaris in a round shape, so named "gelatinous portion" by the author temporarily The author intends to study on its function with an electron microscopic method.

ZUSAMMENFASSUNG

Es wurde darüber berichtet, dass die Otokonien und das Sinnesepithel der Statolithenmembran, der Maculae und der Cristae ampullares vom Frosch mittels eines Raster Elektronenmikroskopes beobachtet wurden. Besonders an der Struktur der Statolithenmembran wurde das Verhältnis zwischen der retikulären Struktur und den Sinneszellen zum ersten Mal festgestellt, was bis jetzt durch elektronische und licht mikroskopische Untersuchungen noch nicht beobachtet werden konnte. Weiter wurde die Morphologie der Sinneszellen ins klare gebracht, ausserdem konnte die Anordnung der Sinneszellen in dem Sinnesepithel und in der Cristae ampullares beobachtet werden. Die Oberflächenstruktur der Übergangszone, des Planum semilunatum und der „gelatinösen Portion" der Cristae ampullares wurde ebenfalls angegeben. Zusätzlich wurde eine Beschreibung der Struktur der „gelatinösen Portion" und der anderen noch nicht beschriebenen Teile hinzugefügt.

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Acta Otolaryng 73 323–334 1972

ELECTRON MICROSCOPIC OBSERVATIONS ON STRUCTURES RELATED TO THE EPITHELIAL BASEMENT MEMBRANE IN SQUAMOUS CELL CARCINOMA

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(Received April 13 1971)

Abstract. Invasive and preinvasive carcinoma of the human oral cavity were examined with attention focused on three structures related to the electron microscopic basement membrane, i.e. half-desmosomes, filaments traversing the lamina lucida, and aperiodic fibrils extending from the basement membrane. These structures were decreased in number or changed in structure in areas where the basement membrane is incomplete or absent. The structural findings were compared to the normal morphology and discussed in relation to the function and origin of the structures. It was found that the layered pattern of the extracellular part of the half-desmosome, reflected in the structure of the individual filament traversing the lamina lucida, is a result of the lateral aggregation of traversing filaments in register Observations support the view that the half desmosomes, in addition to their function as attachment devices, participate in transport of material from the basal cell. Structural and dimensional similarities between artificial SLS-aggregates and native aperiodic fibrils suggest that the latter could be regarded as a tropocollagen aggregate. The aperiodic fibrils are the most prominent structure in the region which, under the light microscope, appears to be composed of reticulin fibres. The reactivity of the aperiodic fibrils with periodic acid-silver methenamine, further supports their relation to the subepithelial argentophil reticulum The possible epithelial origin of the structures described was discussed.

The human oral cavity is covered by a stratified squamous epithelium, which is separated from the connective tissue by a continuous basement membrane (Figs. 1 2) The structure of the basement membrane in carcinoma in situ and invasive carcinoma has been described and compared to the corresponding structure in normal and hyperplastic epithelium in a previous article (Frithiof, 1969) Three types of changes of the basement membrane were recorded in carcinoma in situ and invasive carcinoma. Absence of basement membrane over large areas or an extremely thin basement membrane was the most common observation. Some specimens also included areas with multiple layers of basement membrane and/or areas with an atypical basement membrane material in considerable quantities.

Regarding the substantial changes of the basement membrane it might be of interest to analyse structures associated with the basement membrane such as half-desmosomes, filaments traversing the lamina lucida, and aperiodic fibrils (Figs. 1 3) The changes of these structures in association with different types of basement membrane present in pathological epithelium also give certain hints as far as the functional interpretation of some structural details and their relations are concerned. The present paper aims at describing and discussing these structural features and relations in detail.

This work was supported by the Swedish Cancer Society

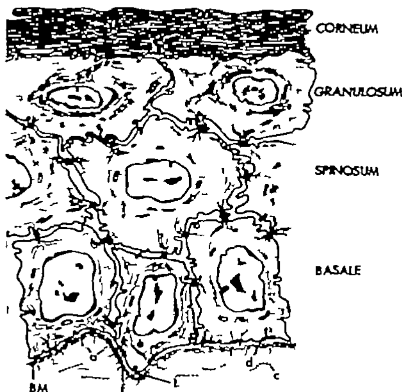


Fig. 1. Outline of keratinized oral epithelium as seen in electron micrographs of human gingiva and hard palate. The number of cell layers is reduced and certain proportions are exaggerated for clarity. *BM* = Basement membrane, *a* = aperiodic fibrils, *f* = traversing filaments, *L* = lamina lucida, *d* half-desmosome, *c* = collagen.

Terminology

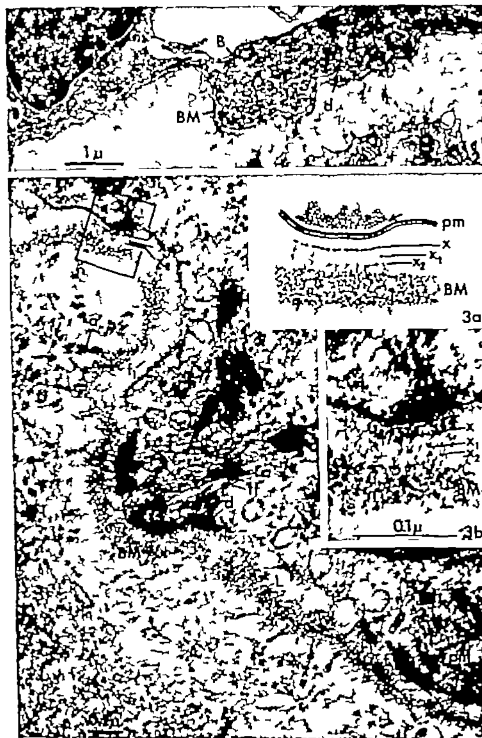
The term "basement membrane" is used as defined by Ottoson et al. (1953) to denote the subepithelial, electron-dense layer about 300–800 Å thick (Figs. 1–3). The basement membrane is separated from the basal cell by a less dense zone of approximately the same width, called the "lamina lucida" (Low 1962). The subepithelial argentophil reticulum, embedded in a homogeneous layer of polysaccharide-containing material seen under the light microscope (Breathnach, 1964; Gersh & Catchpole, 1949) is referred to as the "light microscopic basement membrane".

The term "aperiodic fibril" was suggested (Berghagen & Frithiof, 1966) as a purely morphological term describing the short curved fibril extending from the proximal surface of

Fig. 2. Normal vestibulum. The basal cell (*B*) is separated from the connective tissue by a continuous basement membrane (*BM*). Half-desmosomes (*d*) are recognized as highly dense areas along the plasma membrane. Vestopal. × 15 000.

Fig. 3. Specimen from a pronounced epithelial hyperplasia, with parakeratosis and subepithelial inflammation. The epithelium is regularly ordered and there is no cellular atypia. In the half-desmosome (*d*), bundles of tonofilaments (*tf*) converge towards the vicinity of the plasma membrane. The extracellular components of the half-desmosome include several dense layers (×₁, ×₂) parallel to the plasma membrane (*pm*), and fine filaments (*f*) traversing the lamina lucida (*L*) towards the basement membrane (*BM*). Between the half-desmosomes, the fine structure of the individual traversing filament (*f*) may be seen (cf. Figs. 5–7). Aperiodic fibrils (*a*) join to form a "knot" (*arrows*). Vestopal. × 90 000.

Fig. 3 a. Outline of a half-desmosome. The dense cytoplasmic component is separated from the plasma membrane (*pm*) by a less dense interspace (*arrow*).



The extracellular dense structure ( ) appears to be due to the lateral aggregation of traversing filaments in register. Additional, less distinct "layers" (and) are occasionally observed. Neither the plasma membrane nor the basement membrane (*BM*) is thicker within the half-desmosome. Proportions are exaggerated for clarity.

Fig. 3 b. Framed area in Fig. 3. Symbols as in Fig. 3 × 270 000.

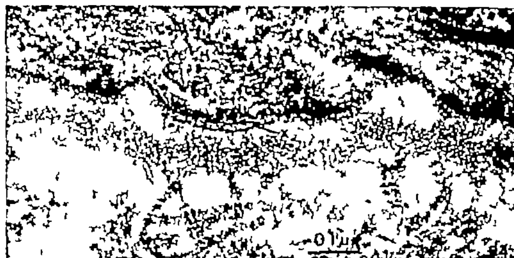


Fig 4 Specimen from an epithelial hyperplasia with degenerative changes and parakeratosis. There is no cellular atypia. The dense intracellular component of the half-desmosome is separated from the plasma membrane by a thin, less dense layer (*arrow*). The extracellular dense layer (×) appears at a constant distance from the peripheral layer of the plasma membrane (*arrowhead*). Epon. × 120 000.

the epidermal basement membrane. This fibril has been described under various names: "reticular" (Brody 1960) "precollagenous" (Frithiof et al., 1964) "lacelike electron dense material" (Melcher 1965) and "anchoring fibril" (Palade & Farquhar 1965 Susi et al. 1967) A term pertaining to structural features is preferred when discussing the chemical composition and function of the fibril.

The term "nonkeratinized" is used here to denote an epithelium which has not developed a stratum corneum The term "keratinized" is used when an ortho- or parakeratotic stratum corneum is present.

MATERIAL AND METHODS

The material consists of 69 specimens, 21 from normal and 48 from pathological oral surface epithelium.[1] The normal material was excised from patients without any clinical signs of affection of the mucous membrane Gingival specimens were taken from individuals with a clinically healthy gingiva, without periodontal pockets or any other clinical signs of periodontal disease. The pathological material includes 25 specimens of epithelial hyperplasia, 13 specimens of carcinoma in situ and suspected carcinoma in situ and 10 specimens of invasive squamous cell carcinoma. Various degrees of differentiation were represented in the last group. The diagnoses[2] were made under the light microscope on paraffin-embedded material and verified on 1 μ thick Epon or Vestopal sections from the same surface as the thin sections used for electron microscopy

Excised specimens were cut in two pieces which were immediately fixed one in a neutral buffered 7% formaldehyde solution for light microscopy and the other in a cold, buffered 1% osmium tetroxide solution (Rhodin, 1954) for electron microscopy Specimens for electron microscopy were dehydrated in ethanol or acetone, and embedded in Epon and Vestopal respectively They were sectioned with an Ultro-

[1] The material has been collected at the Department of Otolaryngology Karolinska Sjukhuset and the Department of Oral Surgery Faculty of Dentistry Karolinska Institutet, Stockholm.

[2] Normal specimens have been checked by Associate Professor Bengt Lagerlöf at the Institution of Pathology Karolinska Sjukhuset. The pathological material was classified in cooperation with Associate Professor Gunnar Moberger at the Institution of Tumour Pathology Karolinska Sjukhuset. A detailed account of the diagnostic criteria was given in a previous publication (Frithiof, 1969).

tome (LKB). Thin sections, stained in solutions of uranyl acetate (Watson, 1958) and lead acetate (Karnovsky 1961) were studied in a Siemens Elmiskop I. Gevaert Litholine or Scientia plates were used. Measurements were made on glossy prints with a magnifying glass (×8) focused on a scale graded in 0.1 mm. Five additional specimens of gingival epithelium were fixed in a buffered 3% glutaraldehyde solution for 1–3 hours and post fixed in 1% osmium tetroxide for 1 hour They were embedded in Epon and examined in stained and unstained thin sections.

All observations are based on specimens fixed in osmium tetroxide solution. Specimens fixed in glutaraldehyde were included for comparison, to exclude that the observations are dependent upon the use of osmium tetroxide as a primary fixative.

OBSERVATIONS

Concerning the structures described in the following there are no differences between specimens from normal and hyperplastic epithelium. Each observation made in one of these two groups of specimens was also made in the other To make up for the absence of a separate description of the latter group and to support the similarity claimed, some of the illustrations were selected from hyperplastic specimens.

Half-desmosomes traversing filaments and aperiodic fibrils are identified also in the specimens fixed in glutaraldehyde. No further reference to these specimens is made.

Half-desmosomes

Normal material. There are no differences in concentration of the half-desmosomes in keratinized and in nonkeratinized epithelium.

At moderate magnification the half-desmosomes are seen as highly dense aggregates along the basal cell surface facing the basement membrane (Fig. 2). They frequently appear as slightly curved prominences of the plasma membrane.

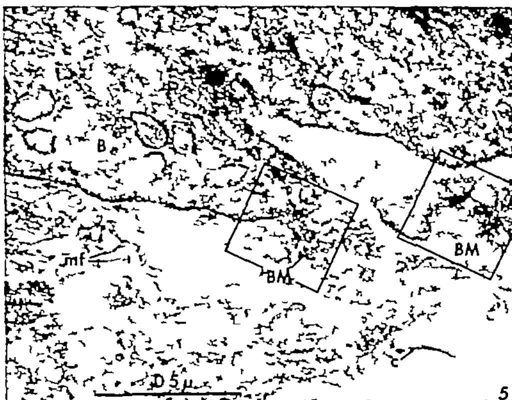
At high resolution the well-known asymmetric, triple-layered plasma membrane is clearly identified, especially within the half desmosomes (Fig. 3) The basement membrane does not appear thicker and no increased density of it is observed opposite the half-desmosomes.

The intracellular part of the half-desmosome is an aggregate of a dense material which is less dense than the cytoplasmic layer of the plasma membrane and is, in many specimens, observed to be separated from the latter by an extremely thin layer of low density (Fig. 4) The dense aggregate is in places divided in two or three parts, that which is situated close to the plasma membrane being then the largest and the one situated deepest in the cytoplasm being the smallest. Tonofilaments are frequently observed oriented as to converge towards the intracellular part of the half-desmosomes.

The extracellular part of the half-desmosome is composed of a 60–90 Å thick layer of a dense, granular material, situated at a distance of about 50 Å from the peripheral layer of the plasma membrane (Fig. 3 *a* ×) A second, less distinct, dense layer (Fig. 3 *a* $\times_1$) is separated from the first by a less dense space, about 60 Å thick. A third, discontinuous layer is occasionally visualized close to the basement membrane (Fig. 3 *a* $\times_2$) Perpendicularly through these layers, numerous thin filaments traverse the lamina lucida and extend into the basement membrane.

Squamous cell carcinoma and carcinoma in situ

In areas where the basement membrane is normal, the half-desmosomes are also normal in structure. In areas where the basement membrane is absent, the half-desmosomes are also absent (Fig. 5). There is then no orientation of tonofilaments in relation to the plasma membrane. In the border zone of areas where the basement membrane is absent, an extremely thin and discontinuous basement membrane is seen. Extracellular and intracellular aggregates of dense material forming half-desmosomes of reduced size (Figs. 5–7) are observed opposite



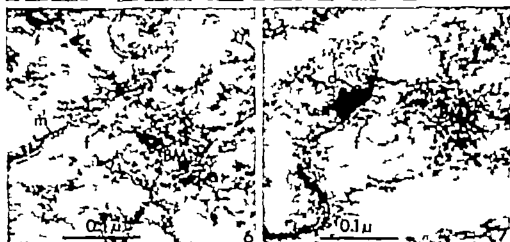


Fig. 5. Specimen from a moderately differentiated, slightly keratinized, invasive squamous cell carcinoma. Absence of a continuous basement membrane in the selected area is noticed. The basal cell (*B*) is separated from the loose network of beaded filaments ("microfibrils") (*mf*) in the connective tissue, by a less dense layer (*arrows*). Reduced half-desmosomes are situated opposite islands of basement-membrane material (*BM*). *c* = Collagen. Vestopal. × 72 000.

Figs. 6 and 7. Framed areas in Fig. 5. Reduced half-desmosomes (*d*) are present opposite islands of basement-membrane material (*BM*). From the triple-layered plasma membrane (*pm*), traversing filaments (*f*) extend into the basement-membrane islands. The transverse pattern of the traversing filaments obviously accounts for the multi-layered appearance of the extracellular part of the half-desmosome. Vestopal. × 192 000.

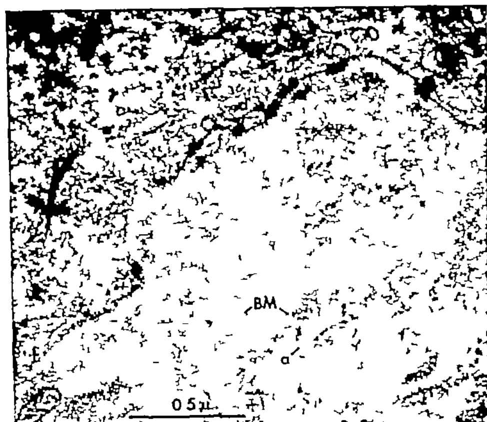


Fig 8 Specimen from a moderately differentiated and slightly keratinized invasive squamous cell carcinoma. Multiple layers of basement membranes (*BM*) are present. Normal half-desmosomes (*d*) are situated in areas where the basement membrane is properly related to the plasma membrane. *a* – Aperiodic fibrils, *f* – traversing filaments. Epon. × 60 000.

even the slightest trace of a basement membrane correctly related to the basal cell. The *half-desmosomes are normal in structure* in basal cells which are associated with multiple basement membranes (Fig. 8). In areas where an atypical basement membrane material is normally related to the plasma membrane, the half-desmosomes appear normal or reduced in size (Fig. 9). In some places where the atypical basement membrane is either closely related to the plasma membrane, in the absence of the lamina lucida, or separated more than normally from the plasma membrane no half desmosomes are to be found (Fig. 9).

Traversing filaments

Normal material Fine filaments—less than 50 A thick—traverse the lamina lucida and extend into the basement membrane where they cannot be distinguished from the filamentous component of the basement membrane. Some of them are branched (Fig. 3 f_1) and closely connected to the peripheral layer of the plasma membrane (Figs. 3 4) The traversing filaments are most numerous within the half-desmosomes. In ideal sections of half-desmosomes in Vestopal-embedded specimens, the aggregation and lateral array of traversing filaments give prominence to an aperiodic and asymmetric pattern in the filaments (Fig. 3) which in the individual filament is seen as at least two dense nodules appearing at a distance of about 50 A and 170 A from the plasma membrane (Fig. 3 f_1). These distances agree with the position of the first and the second extracellular layers of dense granular material, situated at a dis-



Fig. 9. Invasive squamous cell carcinoma with atypical basement membrane material (*BM*). Half-desmosomes are normal (*d*) or decreased in size (*d'*). Half-desmosomes are absent in areas where the basement-membrane material is not properly related to the basal cell (*B*). Epon. × 30 000.

nce of 50 A and 170–200 A respectively from the peripheral layer of the plasma membrane in the half-desmosomes (Fig. 3).

Squamous cell carcinoma and carcinoma in situ

Absence of the basement membrane, as in certain specimens from invasive and preinvasive carcinoma, is accompanied by absence of the traversing filaments. The peripheral layer of the basal plasma membrane is free from filamentous appendages. Fine, beaded filaments and fibrils in the loose sub-epithelial tissue seem in some places to be separated from the basal cell by a less dense space of almost constant thickness (100–130 A) (Fig. 5). Within areas where a continuous basement membrane is absent, small tufts of filaments are identified as an imperfect basement membrane material. The structure of the individual traversing filament is most evident in such imperfect half-desmosomes (Figs. 6, 7).

In areas with multiple basement membranes there is no obvious deviation from normality in the traversing filaments (Fig. 8).

The atypical basement membrane material (Fig. 9) is usually seen in the presence of half-desmosomes, including traversing filaments. In areas where half-desmosomes are absent, the atypical basement membrane material is either in close contact with the plasma membrane—in absence of the lamina lucida—or irregularly separated from it. Here the traversing filaments are absent.

Aperiodic fibrils

Normal material. From the proximal surface of the basement membrane curved fibrils of varying thickness (100–600 A) project towards the connective tissue, frequently forming a distinct fibrillar zone about $^1/_3$–$^1/_2$ μ thick. Favourably sectioned fibrils show a series of dense and less dense, transverse bands arranged in a certain order of succession in relation to the basement membrane. The pattern is neither symmetric nor repeated within the single fibril (Fig. 3) The distal end of the fibril—connected to the basement membrane—consists of fine filaments entering into the fibril separately or in bundles. Bundles can be identified also in other parts of the fibril. Neighbouring fibrils occasionally converge to form a "knot" at some structurally similar point on the fibrils (Fig. 3). There is no obvious, regular connection with collagen fibrils. The latter however are frequently seen within the zone of aperiodic fibrils, sometimes also in contact with the basement membrane.

Squamous cell carcinoma and carcinoma in situ

In places where there is no continuous basement membrane, the aperiodic fibrils are absent and the subepithelial collagen is reduced markedly The same pattern is seen when an extremely thin and discontinuous basement membrane is present. In the subepithelial tissue there are large electron-lucent areas with filaments about 70 A in diameter tube-like in cross section (Fig. 5) and having a beaded or unspecified transverse pattern.

The aperiodic fibrils are regularly present in association with multiple basement membranes, mainly concentrated on the proximal surface of the profound basement membrane layer which is in most places the thickest. Aperiodic fibrils associated with the distal surface of the basement membrane are rarely seen. Thin aperiodic fibrils are associated with the interjacent and distal basement membrane layers.

The aperiodic fibrils do not appear in the presence of the atypical basement membrane material. Collagen fibrils are not frequently present.

DISCUSSION

Half-desmosomes

Desmosomes and half-desmosomes are attachment devices (Hibbs & Clark, 1959 Odland, 1958 Ottoson et al., 1953 Porter 1956 Selby 1955 Weiss & Ferris, 1954) Circumstantial evidences hint at additional functions. Weiss & Ferris (1954) suggested that half-desmosomes are related to fibrogenesis. The active and porous character of desmosomes and half desmosomes, permitting entry of extracellular material and a mutual interaction between the cell and its environment, has been mentioned (Mercer 1961 Weiss, 1958) The more elaborate model of membrane function (Weiss, 1960) which explains the formation of a microbreach of the cell boundary as a reorientation of long molecules from "barrier positions" within the surface to "open gate positions" perpendicular to the surface is recalled when the abundance of fine filaments extending from the plasma membrane to the basement membrane within the half-desmosomes is regarded. Berger & Hundeiker (1967) suggested that the half desmosomes, to a certain degree, are involved in the formation of the basement membrane. Obviously the presence and size of half-desmosomes and the condition of the epithelial basement membrane are related in the present material, as demonstrated in invasive carcinoma and carcinoma in situ.

If it is accepted that the basement membrane is of epithelial origin (Pierce, 1965 Pierce et al., 1964) it might also be justified to regard the filaments traversing the lamina lucida as an epithelial product. The specific accumulation of traversing filaments within the half-desmosomes is therefore an indirect support of the porous spot theory" (Weiss, 1958)

Traversing filaments

According to the previous discussion, the fine filaments situated between the basal cell and the basement membrane (Breathnach, 1964 Kahl & Pearson, 1967 Kobayasi, 1961 Palade & Farquhar 1965 Stern, 1965 Thilander &

Bloom, 1968 Younes et al., 1965) might be regarded as an epithelial product. No continuity between the filaments traversing the lamina lucida and cytoplasmic filaments or aperiodic fibrils (Susi, 1969 Susi et al., 1967) could be demonstrated.

Brody (1968) found, parallel to the prominent extracellular dense layer of the half-desmosomes, additional, less conspicuous layered structures.

The present observations demonstrate that the traversing filament is structurally asymmetric and positionally polarized in relation to the plasma membrane. In the half-desmosome this structural pattern is intensified by their aggregation and lateral array so that the extracellular layered pattern is formed.

Similarly to most thread-like structures observed under the electron microscope the traversing filaments have been assumed to serve as an attachment device. Experimental blistering of human skin with suction, demonstrates that the weakest point in the attachment system between the dermis and the epidermis is the lamina lucida the half-desmosomes being the last part of the basal cell to lose contact with the basement membrane (Kiistala & Mustakallio 1967) It is interesting to note the obvious [illegible] of the extracellular layered pattern of the half-desmosomes in 4 cases of epidermolysis bullosa hereditaria letalis, described by Arwill et al. (1968) as one of several profound structural deviations from normality

Aperiodic fibrils

Divergent views upon the composition of the aperiodic fibril have been expressed. Selby (1955) concluded that the fibrils "must correspond to the argyrophilic fibers of the argyrophilic reticulum described by histologists in this region" She pointed out that the finer argyrophilic connective tissue fibres of the upper margin of the dermis are basically young collagen (reticulin), with a particular affinity for polysaccharides. Menefee (1957) held a similar opinion. Observations by Berger & Hundeiker (1967) Swift & Saxton (1967) and Younes et al. (1965) based on Movat's (1961) periodic acid-silver methenamine (PASM) method to stain glycoproteins in thin sections for electron microscopy support essential parts of Selby's discussion. Palade & Farquhar (1965) on the other hand, who were the first to clearly demonstrate the unique aperiodic pattern in detail, concluded that the identification of the aperiodic fibril as "reticulin" is unlikely because of its dimensions, distribution and structure. Morphological studies on "reticulin" have demonstrated a structural periodicity of about 650 A (Gross, 1950; Little & Kramer 1952).

This divergence may be explained by the fact that several types of tropocollagen aggregates exist (Hodge, 1960 Olsen, 1967), and the possibility that more than one of these may be argyrophilic and react with PASM. Special attention should be paid to the SLS-types of artificial fibrils formed by lateral aggregation of tropocollagen (Hodge, 1960· Olsen, 1967). The length of the artificial fibrils is within the same range of size (about 0.25 micron) as the native aperiodic fibrils, and they have also a similar aperiodic and asymmetric pattern of transverse bands of varying density (Rowlatt, 1969) Symmetrically paired aggregates of aperiodic fibrils, observed in the tail of tadpoles, have been compared structurally with the vitreous bodies, composed of tropocollagen (Bruns, 1969).

Even though complete structural identity has not been established between the native aperiodic fibrils and any aperiodic tropocollagen aggregates, the aperiodic structure no longer serves as an argument against the conception that tropocollagen is included in the content of the aperiodic fibril.

The origin of the aperiodic fibril is not known. In the present material they were only seen associated with well developed basement membranes. They are present also adjacent to basement membranes separated from the connective tissue by one or more layers of additional (older) basement membranes. Aperiodic fibrils associated with large amounts of base-

ment membrane material fill up the acellular areas in adenoid cystic carcinoma of the cribriform type (Eneroth et al., 1968) In the acellular areas, which are surrounded by epithelial cells, there are no connective tissue cells or classical collagen fibrils (Eneroth et al., 1968) The findings in adenoid cystic carcinoma and the observations described in this paper make it apparent that the possibility that the aperiodic fibrils and the basement membrane material have a common epithelial origin should not be disregarded. Hay (1964) suggested the possibility that a material containing hydroxyproline is transferred from the basal cell to the connective tissue.

The subepithelial electron lucent areas, observed in invasive and preinvasive carcinoma, associated with a reduced basement membrane and absence of aperiodic fibrils and collagen, are regarded as fluid spaces. Fine filaments resembling microfibrils (Haust, 1965 Low 1961) supposed to be simple aggregates of tropocollagen (Haust, 1965) are frequently the only structural element identified in such areas.

ACKNOWLEDGMENT

This study has been made possible by the generous support and never failing interest of Associate Professor Jan Wersäll, to whom I express my sincere gratitude.

ZUSAMMENFASSUNG

In der vorliegenden Untersuchung von invasiven und präinvasiven Karzinomen in der menschlichen Mundhöhle werden folgende drei mit der elektronen-mikroskopisch definierten Basalmembran in Zusammenhang stehende Strukturen einem näheren Studium unterzogen: die Halb-Desmosomen, die die lamina lucida quer durchlaufenden Filamente und die von der Basalmembran ausgehenden aperiodischen Fibrillen. Diese Strukturen zeigten in den Zonen, in denen die Basalmembran inkomplett ist oder fehlt, eine zahlenmässige Abnahme oder strukturelle Veränderung. Die beobachteten Befunde werden mit der normalen Morphologie dieser Strukturen verglichen und in Hinblick auf Funktion und Ursprung dieser Strukturen diskutiert. Wie es sich zeigte, resultiert das Schichtungsgefüge des extrazellulären Teils der Halb-Desmosomen, das in der Struktur der die lamina lucida quer durchlaufenden Einzelfilaments wiederzuerkennen ist, aus der lateralen Aggregation der in einer systematischen Struktur angeordneten durchlaufenden Filamente. Die Beobachtungen stützen weiter die Anschauung, dass die Halb-Desmosomen zusätzlich zu ihrer Funktion als Bindestruktur am Materialtransport von der Basalzelle beteiligt sind. Ähnlichkeiten in Struktur und Dimension zwischen artifiziellen SLS-Aggregaten und gewebeeigenen aperiodischen Fibrillen deuten auf die Möglichkeit, dass es sich bei den letzteren um ein Tropokollagenaggregat handelt. In der Zone, die im Lichtmikroskop aus „Retikulinfibern" zusammengesetzt zu sein scheint, sind die aperiodischen Fibrillen die am stärksten hervortretende Struktur. Die Reaktion der aperiodischen Fibrillen auf perjodsaures Silbermethenamin ist eine weitere Stütze eines Zusammenhanges dieser Fibrillen mit dem subepithelialen „argentophilen Retikulum". Abschliessend wird die Möglichkeit, dass sich die beschriebenen Strukturen aus dem Epithel entwickeln, diskutiert.

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Acta Otolaryng 73 335–340, 1972

INFLUENCE OF CHRONIC TREATMENT WITH NEOMYCIN AND KANAMYCIN ON N_1 SUPPRESSION PRODUCED BY CONTRALATERAL OLIVO-COCHLEAR BUNDLE STIMULATION IN CATS

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(Received April 13 1971)

Abstract. Twenty cats received one subcutaneous injection per day of neomycin sulfate (100 mg/kg/day) and 5 kanamycin sulfate (200 mg/kg/day) for 8 days; experiments were performed on the ninth day Nine of 10 untreated control, 13 of 20 neomycin and 5 of 5 kanamycin animals had normal cochlear responses to acoustic stimulation and were usable for this study The efficacy of contralateral olivo-cochlear bundle (COCB) stimulation was determined at 400 (optimally effective) and 100 (minimally effective) Hz for 4 different current strengths at each frequency of stimulation. Six of the neomycin animals exhibited a significant block of COCB stimulation effects at all currents and both frequencies of stimulation, while the other 7 neomycin animals and the kanamycin animals showed a significant increase in the effectiveness of COCB stimulation at the lower currents of the higher (optimal) stimulation frequency Thus, chronic treatment with neomycin and kanamycin produces changes in COCB stimulation effects, indicating that alterations of cochlear efferent function may be playing a role in the genesis of the ototoxicity produced by certain *Streptomyces* antibiotics.

Kohonen (1965) found that the pattern of hair cell degeneration produced by neomycin and kanamycin in guinea pigs paralleled the degree of density of the efferent innervation in the cochlea. He suggested that their ototoxic effects may be due to interference with efferent function.

Brown (1968) using cats, found that acute intra-arterial administration of neomycin, kanamycin and streptomycin produced no changes in the N_1 suppression and microphonic augmentation obtained by stimulating the contralateral cochlear efferents (contralateral olivo-cochlear bundle, COCB). He did, however find that an occasional animal responded with either an increase in microphonics, a depression of N_1 or both that seemed to be causally related to the injection of the ototoxic agent. In addition, N_1 depression and microphonic augmentation were obtained by Sohmer & Feinmesser (1965) after streptomycin and dihydrostreptomycin were iontophoresed into the cochlea of guinea pigs and increases in microphonics were produced by neomycin, kanamycin, streptomycin and dihydrostreptomycin after being injected peritoneally into cats (Blair Simmons et al., 1960). Therefore, the ototoxic antibiotics appear to exert a weak agonistic action at the efferent synapses when injected acutely

The purpose of this study was to determine if chronic treatment with neomycin and kanamycin has any effect on COCB stimulation.

This research was supported in part by a grant from the Southern Medical Association.

Presented at the annual meeting of the Federation of American Societies for Experimental Biology and Medicine April 1971

METHODS

Thirty-five healthy adult cats, 12 males and 23 females, weighing between 1.9 and 5.2 kg were used. The animals were anesthetized with intraperitoneal injections of Dial-Urethane, 0.65 ml/kg for the untreated control animals and 0.5 to 0.6 ml/kg for animals pretreated chronically with neomycin and kanamycin. Smaller doses had to be used in the animals treated with neomycin and kanamycin as they were in relatively poor condition as a result of varying degrees of neuromuscular blockade and disturbances of vestibular function produced by the antibiotics.

Animals were prepared for round window recording and stimulation of the COCB in a manner similar to that described by Brown et al. (1969).

N_1 suppression was chosen as the criterion by which to gauge the efficacy of COCB stimulation rather than microphonic augmentation as changes in N_1 are much greater than the changes in microphonics produced by COCB stimulation (Desmedt & Monaco, 1961). In addition, in a previous study evaluating the effects of selected cholinergic drugs and strychnine on COCB stimulation effects (Brown et al., 1969), analysis of microphonics yielded no information that was not obtainable from analysis of N_1.

The intensity of the acoustic stimulus was varied at the beginning of each experiment in order to obtain an input-output function of N_1. To do this, N_1 height (output) was measured for various intensities of the acoustic stimulus (input). (Input was converted from voltage to dB by using as arbitrary reference, 1.0 V = 0.0 dB.) The intensity of the click was then set at least 35 dB above the visible threshold of N_1 for each cat. This intensity was maintained throughout the rest of the experiment. Using the N_1 input-output functions, data of the various COCB stimulations were accumulated on the N_1 suppressions in terms of equivalent decibels attenua-

Table 1. *Equivalent decibels attenuation of N_1 produced by COCB stimulation*

Category	Mean dB attenuation ±S.E.M. (no. of animals)							
	400 cps on S_1				100 cps on S_1			
	Weakest mA	Weak mA	Strong mA	Strongest mA	Weakest mA	Weak mA	Strong mA	Strongest mA
Controls	8.31±1.73 (9)	16.8±5.15 (9)	18.6±1.06 (9)	19.2±1.60 (8)	5.67±1.44 (9)	10.7±1.35 (9)	13.5±0.598 (9)	12.4±1.06 (8)
Neomycin, blocked	2.22±0.903 (6) $p<0.01$	5.40±2.37 (4) $p<0.005$	6.80±2.62 (4) $p<0.005$	7.17±1.76 (6) $p<0.005$	1.75±0.722 (6) $p<0.05$	3.45±1.54 (4) $p<0.005$	4.13±1.19 (4) $p<0.005$	3.92±1.36 (6) $p<0.005$
Neomycin, no block	16.3±2.38 (7) $p<0.01$	21.1±1.18 (7) $p<0.005$	20.7±2.15 (7) $p<0.20$	21.4±2.36 (7) $p<0.30$	8.83±2.05 (7) $p<0.20$	11.8±1.25 (7) $p<0.30$	12.7±1.66 (7) $p<0.40$	12.8±1.62 (7) $p<0.40$
Kanamycin	14.8±1.76 (5) $p<0.025$	21.4±3.70 (5) $p<0.10$ (very close to 0.05)	24.7±2.77 (5) $p<0.025$	23.8±2.71 (5) $p<0.10$	7.07±1.45 (5) $p<0.30$	13.3±1.75 (5) $p<0.20$	13.8±2.28 (5) $p<0.40$	14.8±2.57 (5) $p<0.20$

All p values obtained by doing unpaired Student t-tests comparing the control dB attenuations to the others of the same column.

Table II. *COCB stimulating currents*

Category	Mean current ± S.E.M. (no. of animals)			
	Weakest mA (8 V)	Weak mA (15 V)	Strong mA (30 V)	Strongest mA (40–45 V)
Controls	0.711 ± 0.046 (9)	1.53 ± 0.182 (9)	3.60 ± 0.344 (9)	5.93 ± 0.922 (8)[a]
Neomycin, blocked	0.692 ± 0.080 (6)[b]	1.38 ± 0.253 (4)[c]	2.93 ± 0.671 (4)[c]	5.03 ± 1.08 (6)
Neomycin, no block	0.636 ± 0.067 (7)	1.48 ± 0.193 (7)	3.63 ± 0.619 (7)	5.69 ± 1.03 (7)
Kanamycin	0.620 ± 0.058 (5)	1.68 ± 0.289 (5)	3.80 ± 0.891 (5)	5.88 ± 1.37 (5)

[a] Unable to perform stimulation at this setting on one of the nine control animals because of technical problem with equipment.
[b] The control currents are not significantly different from the currents obtained from any of the other categories.
[c] Did not perform stimulation at these settings on two of the six neomycin, blocked animals.

tion of the sound energy (Desmedt & Monaco 1961).

Efficacy of COCB stimulation was determined by ascertaining the amount of N_1 suppression produced at 400 and 100 Hz of stimulation, using 8 15 30 and 40–45 V of stimulation at each frequency setting.

Statistical analyses were performed using either two-tailed, unpaired Student *t* tests (as described by Dixon & Massey 1957) or tests of significance on enumeration data when cell frequencies are small (Fisher's exact test as described by Batson, 1956).

RESULTS

Twenty cats received 1 subcutaneous injection per day of neomycin sulfate[1] (100 mg/kg/day) and 5 kanamycin sulfate[2] (200 mg/kg/day) for 8 days followed by determination of the efficacy of COCB stimulation on the ninth day. Chronic treatment with this neomycin dosage schedule has been demonstrated to produce both cochlear and vestibular damage in cats (Blair Simmons et al. 1960); the kanamycin dosage schedule was based on extrapolations from the data of Daigneault et al. (1970) and Pruett (1968). Ten untreated cats served as controls. The dates of experiments on the various series of animals were interspersed over a 4 month period (Table IV).

Efficacy of COCB stimulation could not be determined on 8 of the 35 animals, however. One of the controls had a distorted N_1 input output function in that microphonics began at a lower intensity acoustic stimulus than N_1 did and rode into and interfered with the interpretation of N_1 amplitudes, 1 of the neomycin animals died on the seventh day of injection from respiratory paralysis presumably caused by the neomycin, and 6 other neomycin animals had no recordable N_1 and very small or no microphonics on the day of COCB stimulation (these animals also showed more respiratory depression than the usable neomycin animals did). Therefore, evaluation of the efficacy of COCB stimulation was carried out on 9 of 10 control animals, 13 of 20 neomycin animals and 5 of 5 kanamycin animals.

The equivalent decibels attenuation of N_1 obtained at 400 and 100 Hz with the 4 currents of COCB stimulation are presented in Table I. Table II depicts the currents of COCB stimulation obtained at the various voltages of stimulation.

In 6 of the usable neomycin animals, the most effective COCB stimulation for each

[1] Sigma Chemical Co. (pfs), St. Louis, Missouri, Lot no. 059 B 2130.
[2] Sigma Chemical Co. (pfs), St. Louis, Missouri, Lot no. 030 C 2560.

Table III *Incidence of blocked COCB effects in control and neomycin-treated animals*[a]

Category	Block	No Block	Totals
Usable Controls	0	9	9
Usable Neomycin Animals	6	7	13
Totals	6	16	22

Calculation of exact probability for the tail the observations are on yields $p = 0.02300$. For both tails, $p = 0.046094$

animal was less effective than those of any of the control animals (the neomycin, blocked category). These animals were placed in a separate category because use of Fisher's exact test yielded a two-tailed probability of less than 0.05 (Table III) indicating that the block seen in these animals did not occur by chance or result from poor placement of the stereotactic electrode. In the other 7 usable neomycin cats, the most effective COCB stimulation was either more effective than or the range of the controls (the neomycin, block category) The 5 kanamycin animals also fell into this category One of the neomycin animals in the no block category and 2 of the kanamycin animals had COCB stimulation effects greater than those of the controls. However these animals were left in the no block category because use of Fisher's exact test yielded one-tail probabilities of much greater than 0.05 indicating that the effects could have occurred by chance or resulted from better placement of the stereotactic electrode

As shown in Table I the neomycin, blocked category exhibited a significant reduction in effectiveness of COCB stimulation at all 4 currents and both frequencies of stimulation On the other hand, the neomycin no block category and the kanamycin animals were found to have a significant increase in the effectiveness of COCB stimulation at the lower currents of stimulation of the higher (optimal) stimulation frequency

The data on weight (grossly related to age), sex, dates of COCB stimulation, dB of the acoustic stimulus used when stimulating the COCB and N_1 height at this acoustic stimulus and at 0 dB were compiled (Table IV) in order to determine if these variables were responsible for or related to the animals falling into the various categories.

There was no significant difference in the dB intensity of the acoustic stimulus used when stimulating the COCB or in the N_1 height at that intensity indicating that no large amount of damage had occurred to the ears of the usable neomycin animals and the kanamycin animals. An attempt was made to compare the control *before stimulation* amplitudes of N_1 and microphonics with the *neomycin* and kanamycin *before stimulation* amplitudes. However the hollow ear bars attenuated the click to a different extent from preparation to preparation Because of this, a normal distribution of the different input-output functions could not be depended on, therefore, a standard acoustic stimulus could not be set at which to compare the amplitudes of the controls with those of the neomycin and kanamycin animals. The N_1 heights at 0 dB were recorded on all of the animals, however and there was no significant difference in these again indicating that a large amount of cochlear damage had not occurred in the antibiotic treated cats.

The unusable neomycin animals obviously had cochlear damage, but it was not possible to determine whether this was due to the ototoxic effects of neomycin, to chronic anoxia caused by the neuromuscular depression produced by neomycin, or to a combination of the two The unusable control had a cochlear *response qualitatively similar to that* obtained by Ronis (1966) from a patient who had otosclerosis therefore, ossicular chain fixation was probably the reason for the distorted pattern obtained in this cat.

Survey of the data on dates of COCB stim-

Table IV *Weight sex dates of COCB stimulation the dB intensity of the acoustic stimulus used when stimulating the COCB N height at this acoustic stimulus and N_1 height at 0 dB*

Category	Weight (mean kg ±S.E.M.)	Sex	Dates of COCB stimulation (month/day of 1970)	Mean dB (acous. stim. ±S.E.M.)	N_1 height (μV) at this acous. stim.	N height (μV) at 0 dB[a] of acous. stim.
Usable controls	3.49±0.384	4 ♂ 5 ♀	6/23 6/25 7/8, 7/9 8/6, 8/25 9/9 9/10, 9/11	+ 8.44±3.77	273±49	134±43
Unusable control	4.38	1 ♂	7/13	—	—	—
Unusable neomycin	2.86±0.256	3 ♂ 4 ♀	6/17 7/13 7/15 8/12, 8/20, 8/21 8/28	—	—	—
Neomycin, blocked	3.09±0.403	2 ♂ 4 ♀	6/16, 6/17 8/13, 8/20 9/4, 9/23	+ 13.7±7.37	319±54	173±48
Neomycin, no block	2.42±0.138*	1 ♂ 6 ♀	7/15 7/17 8/12, 8/13 8/21 8/28, 9/3	+ 12.9±3.32	293±51	170±77
Kanamycin	2.69±0.184*	1 ♂ 4 ♀	6/30, 8/14 8/14 9/2, 9/23	+ 13.6±5.38	279±57	194±58

Neomycin, no block and kanamycin animals are smaller than controls (kanamycin not significant). However there is no significant difference between the neomycin categories.
[a] The only dB common to the input–outputs of all of the animals in the various categories.

ulation leaves one with the conclusion that the different categories of animals do not fall into groupings, indicating that there was no technical error unknowingly repeated by the experimenter for any period of time which caused the results of the antibiotic-treated animals to fall into categories.

DISCUSSION

Chronic treatment with neomycin and kanamycin at doses expected to produce definite ototoxic effects, resulted in modification of the effects of COCB stimulation. Histologic examination of the cochleas of these animals was not performed to determine the extent of ototoxic damage; therefore, it is not possible to state whether the blocked neomycin animals suffered more or less cochlear damage than the other usable neomycin animals and the kanamycin animals did. There was probably no gross difference however as the N_1 input-output functions and the dB intensity of the acoustic stimulus used when stimulating the COCB were not observably different.

Why the dosage schedule of neomycin used in these experiments produces a block of COCB stimulation effects in some animals and an enhancement in others, and the kanamycin dosage schedule produces only an enhancement, is unknown. However a possible explanation could be based on the fact that the ototoxic *Streptomyces* antibiotics accumulate in the cochlear perilymph (Voldrich, 1965 Wersäll & Lundquist, 1968). Thus, neomycin and kanamycin may produce a block of COCB stimulation effects at one range of cochlear concentrations and enhancement at another range of concentrations. The block and enhancement of the usable neomycin-treated animals would then be due to different cochlear concentrations of neomycin being reached because of variations in absorption, distribution, metabolism and/or excretion of the drug by the different animals. The enhancement of COCB stimulation effects produced by kanamycin with no block occurring in any of the animals could be due to the dosage schedule used resulting in the kanamycin cochlear concentrations of all these cats being in the range which produces enhancement. Investigations of chronic treatment with neomycin and kanamycin for less and greater periods of time than in this report are needed to clarify this matter

The apparent agonistic action of neomycin and kanamycin when injected acutely could be related to their mimicking the cochlear efferent mediator or initiating its release. Enhancement or block of the effects produced by COCB stimulation after chronic treatment with these agents could be related to an interference with inactivation of the mediator or occupation of postsynaptic receptors involved in the mediator exerting its effect.

The effects on COCB stimulation of chronic treatment with neomycin and kanamycin could be produced by a cytotoxic action only coincidentally related to the efferent synapse but could also be produced by specific pharmacological actions on the efferent synapse. The well known effect of the *Streptomyces* antibiotics on protein synthesis might very well explain the ototoxic process. However the results of this report suggest that selective effects on the cochlear efferent synapse could also be playing a part in the genesis of ototoxicity

ACKNOWLEDGMENT

I gratefully acknowledge the excellent technical assistance of Mr Tandy W McElwee, Jr

ZUSAMMENFASSUNG

Versuche an Katzen wurden unternommen um festzustellen, ob die Ototoxizität des Neomyzin und Kanamyzin mit einem Einfluss auf die efferente, kochleare Synapse zusammenhängt. Nach chronischer Behandlung mit Neomyzin und Kanamyzin (eine tägliche subcutane Injektion von 100 mg/kg und 200 mg/kg respektive für acht Tage) konnten definitive Veränderungen in der N_1-Depression, verursacht durch Stimullerung des kontralateralen olivo-cochlearen Bündels (KOCB), beobachtet werden. Die mit Neomyzin behandelten Tiere verteilten sich auf Kategorien. 7 Tiere bei denen die N Potentiale nicht auswertbar waren, 6 Tiere bei denen die N Depression, verursacht durch KOCB Stimullerung, blockiert war und 7 Tiere in denen die KOCB Stimullerung unter optimalen Parametern eine grössere N Depression verursachte als bei den Kontrolltieren. Alle mit Kanamyzin behandelten Tiere gehörten zur letzteren Kategorie. Es ist damit klargestellt, dass eine Beziehung zwischen der ototoxischen Wirkung der Antibiotika und der efferenten kochlearen Synapse besteht. Ob diese Beziehungen auf einer unspezifischen, zytotoxischen Wirkung der Antibiotika oder auf einem spezifischen, pharmakologischen Effekt an der efferenten Synapse beruhen, wird in weiteren Untersuchungen bestimmt werden.

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Acta Otolaryng 73 341-343 1972

PSEUDOCALORIC NYSTAGMUS

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(Received June 2, 1971)

Abstract. Testing of the caloric function of the labyrinth is often performed by irrigation with cold water Attention is drawn to pseudocaloric nystagmus, an apparently normal caloric nystagmus, which may be provoked by irrigation with cold water in an ear with abolished vestibular function. It is recommended to perform caloric testing by irrigation with water at 30° and 44°C, but if the purpose is only to determine whether caloric reaction is preserved, the test should be performed with cold water in the prone or with warm water in the supine position.

The function of the labyrinth can be studied by caloric stimulation with water at 30 and 44 C by the method of Hallpike. The patient is examined in the supine position with 30 flexion of the head. The lateral semicircular canal will then be in the vertical position. The adequate stimulus is presumed to be endolymphatic currents set up in the lateral semicircular canal.

The caloric test is sometimes performed only by irrigation with cold water (Wolfson et al., 1965) or in the presence of perforation of the eardrum, by blowing air at room temperature into the ear canal. Done in this way however the test may give directly misleading information as to whether the labyrinth is functioning.

If vestibular function is absent in one ear nystagmus may be provoked by various stimuli. The direction of this nystagmus is always towards the opposite ear This phenomenon is termed pseudocaloric nystagmus.

MATERIAL

During the last 2 years we have seen 8 patients presenting pseudocaloric nystagmus. The patients were 3 men and 5 women aged from 31 to 68 years.

Examination of their ears, nose and throat, and audiometry were carried out. By vestibular examination no spontaneous or positional nystagmus was revealed in any of the patients by means of Frenzel's glasses. In three cases, photo-electronystagmography by the method of Torok was performed, but no nystagmus was demonstrated.

By irrigation with 30 C water (or in one case, with injection of air at room temperature) in the supine position, caloric reaction was induced on both sides, with the direction of the nystagmus away from the stimulated ear By irrigation with 44 water however no reaction was found in one of the ears, and by more pronounced caloric stimulation with water at 4 C and the patient in the prone position with the head tilted 30 forward, no caloric nystagmus towards the stimulated ear could be provoked either The first caloric reaction induced by injection of 30°C water and air at room temperature in the supine position was thus a pseudocaloric nystagmus (Fig. 1). The reaction was of the same or a weaker intensity and shorter duration than that provoked by stimulation of the healthy ear with 44 water

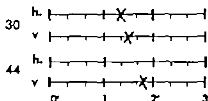


Fig 1 Caloric reactions in a patient with pseudocaloric nystagmus.

The abolished vestibular function was caused by suppurative labyrinthitis, head injury congenital lack of acoustic vestibular function, labyrinthectomy (by the method of Cawthorne) and, in two cases by an acute vascular insult. In two cases, there was no explanation of the loss of vestibular function

DISCUSSION

Decreased activity of one labyrinth may cause nystagmus towards the opposite side (De Kleyn 1939) or may in some cases give a preponderance towards the normal ear without the presence of spontaneous nystagmus. By irrigation with cold water this reaction will interfere with the preponderance and may ...e the induced nystagmus appear normal. ...ation with warm water however will ...use a great difference in the reaction of the two ears (Mittermaier 1965 Henriksson et al. 1970). This is the basis for the recommendation to start all caloric tests with irrigation with warm water

By suddenly abolished function of one labyrinth, nystagmus will be elicited with a direction towards the unaffected ear ("destruction nystagmus") After a period of varying length this nystagmus will often disappear as an expression of a state of compensation. The destruction nystagmus may however be provoked by various sensorial stimuli for example by irrigation with cold or warm water It is, however characteristic that the direction of the nystagmus does not change even if the position of the head is changed (De Kleyn 1939). *The reaction can be explained by the* provocation of a latent nystagmus by vasomotor or by sensory action in the external auditory canal (Grahe, 1927 Jongkees, 1948, 1950)

Some cases of this condition are on record. In these, an apparently normal or near-normal reaction was produced by irrigation with cold water Further examination with changes in the position of the head and irrigation with warm water (surprisingly) revealed that it was a pseudocaloric nystagmus (Lund, 1922, 1924 Grahe, 1923 1927 De Kleyn 1939 Jongkees, 1950 Zwergius, 1954 Everberg, 1960).

In some of the patients in this series, the condition before testing was suggestive of an *affection of the labyrinth, while the absent* caloric reactions in others was an unexpected discovery If in these patients, labyrinthine function had been tested only with cold water in the supine position, the absence of vestibular function on the side concerned would have passed unrecognised.

It is of great importance to bear in mind the possibility of pseudocaloric nystagmus in the evaluation of the function of the labyrinth for example if an acoustic neurinoma is suspected after head injuries, in the testing for residual vestibular function after labyrinthectomy etc.

Pseudocaloric nystagmus can be revealed by irrigation with cold water with the patient in the prone position or with warm water in the supine position.

ZUSAMMENFASSUNG

Die Untersuchung der kalorischen Funktion des Labyrinthes ist manchmal durch Spülung mit kaltem Wasser unternommen worden. Die Aufmerksamkeit wird auf den pseudokalorischen Nystagmus hingelenkt, eine scheinbar normale, kalorische Reaktion. Diese Reaktion wird bei Spülung mit kaltem Wasser in einem Ohr mit erloschener vestibularer Funktion hervorgerufen. Es ist zu empfehlen, die kalorische Untersuchung bei Spülung mit sowohl warmem als auch kaltem Wasser durchzuführen. Wenn man, als Orientierung, nur zu untersuchen wünscht, ob die kalorische Reaktion vorhanden ist, muss die Untersuchung mit kaltem Wasser in Bauchlage oder mit warmem Wasser in Rückenlage *ausgeführt* werden.

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Acta Otolaryng 73 344-352, 1972

NON EXPERIMENTAL AURAL PATHOLOGY IN THE BABOON

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(Received July 12, 1971)

Abstract Among 82 temporal bones of baboons, investigated in histological sectional series, 24 (29.5 percent) exhibited histopathological changes. The latter were tabulated after preceding anatomical remarks given as a help to interpret the morbid anatomy Of surgical consequence, by absence of a subiculum is the open niche of the round window offering direct access to the spaces of the cochlea. This advantage is counteracted by the structure of the external auditory canal which, as an obstacle, must be eliminated to make the deeper regions thus accessible.

Known and described in Pharaonic times (Roth, 1965) the baboon went through a number of classifications. "Anubis" was the Egyptian god represented with a dog head ("Cynocephalos"). Seemingly it was Erxleben (1777) who decided on the generic distinction "Papio" In the systematic list of Hill (1967) the genus Papio comprises the species *Papio anubis* (Cuvier 1823) and *Papio ursinus* (Kerr 1792) the animals which were the objects of the present investigation. Sebeok (1968) remarked that older literature indicated a far more extensive taxonomic splitting than can now be substantiated.

Care and management of the baboon was outlined by England (1953) among others. Interest in the baboon as a research animal is increasing, as emphasized by Vice et al. (1966) who gave directions for the care and raising of newborn baboons.

This work was supported by grants from. The Research Fund of the American Otological Society Inc. Deafness Research Foundation, Research Study Club of Los Angeles, Inc.

Unlike diseases of other organs of primates, aural pathology did not receive deserved attention. This was the motivation to collect and publish data in two other even more popular experimental primates: squirrel monkeys (marmosets) and rhesus (Kelemen, 1966, 1968) The baboon is becoming the third most frequently used primate. It is hoped by these investigations to reduce erroneous interpretations, especially in innumerable experiments around the inner ear One of those who postulated more knowledge on the role of latent infections in the overall health of research animals, was Hummer (1967). Otherwise complete confidence cannot be placed in the results obtained from the primates.

MATERIAL AND METHODS

Specimens were received from different sources in the form of the entire cranial base. The temporal bones were removed and decalcified with Versene (Tetrasodium edetate), serially sectioned (25–30 μm) predominantly in the horizontal plane, stained with hematoxylin-eosin and some auxiliary stains, and were examined and photographed in full and in polarized light. If not marked otherwise the illustrations represent hematoxylin-eosin pictures.

The data supplied varied according to the source. The following obliged with their generous help: Professor H. W Weber Head of the Department of Pathology University of









Fig 1 External canal made tortuous by protrusions of its wall. × 9

Fig 2 Tubal cartilage reaching tympanic cavity × 16.

Fig 3 Subarcuate fossa, lumina of semicircular canals. × 8.

Fig 4 Sequestration in middle ear; "tripartite" incudo-malleolar articulation, produced by tortuosity of the ossicular contours. × 4

Stellenbosch, Belleville, South Africa, sent the petrous bones of 26 baboons which had been trapped and transported to his institute. Of these 19 were tabulated after a stay in the colony of 29.5 days on average. Their average weight was 26 pounds (11.86 kg). 6 were males, 13 females; their ages, of course, were unknown. In 14 the blood groups were de

termined they were A (3 subjects), AB (5) B (6) According to Professor Weber "they belonged to the species Papio ursinus, a Chacma baboon which inhabits most of South Africa and especially the southern parts" Dr B A. Kerr from the Regional Primate Research Center (Director Professor T C. Ruch, Ph D) University of Washington Seattle Washington sent 16 specimens of *Papio anubis* all males, aged between 4 and 6 years, with weights between 9 7 and 11 1 kg. Professor Allan A. Katzberg, Ph.D., Chairman of the Department of Anatomy Southwest Foundation for Research and Education San Antonio Texas, helped with 3 specimens, two 5-pound and one 3-pound male respectively Coming from different sources and with different descriptions, no unified tabulation of the entire material of 45 animals has been possible. 82 ears were prepared in sectional series.

The histological work was done by Mrs Agnes Ward, A.R.C.P and Mrs Ruth Greulich the photography by Mr Lloyd Matlov-

ANATOMICAL REMARKS

As no systematic description is planned certain peculiarities will be merely pointed out as an aid in interpreting some of the pathological findings. Morphological problems around the development of the skull base have been treated by Starck (1965) a mosaic-like pattern of growth of functional components is maintained and preserves some autonomic tendencies. Schultz (1956) treated the development of the mastoid processes. He found (1956) that "the face grows forward to a marked degree" This movement might influence the course of the external auditory canal.

External ear

The narrow tortuous external auditory canal (Fig. 1) does not run horizontally as in humans, but bends downward in a sharp angle. Werner (1960) in *Papio maimon* L. found the canal to be inclined strongly backward (Schultz, 1956) Meyer (1931) had no success with otoscopy in spite of specially manufactured ear specula In experimentation he suggested shortening and artificially opening the canal. The straight and short *Eustachian tube* reaches with its cartilage into the tympanic orifice giving the latter the aspect of a cartilaginous funnel (Fig 2) Its lumen was empty in all observed cases.

Middle ear

Meyer found no real *mastoid process*. It remains restricted to the contours of the osseous cranium without becoming prominent. Haag (1933) in *Cynocephalus hamadryas* found it very flat lying practically in the plane of the occipital bone. Beck (1935) called it rudimentary in primates, and becoming clearly marked only in anthropoids.

The subarcuate fossa (Fig. 3) according to Haag, reaches the same depth as the internal acoustic meatus. The course of the subarcuate artery can be followed, as in humans (Kelemen 1970) crossing in its osseous canal between the limbs of the arch of the superior semicircular canal and forming afterward, in a single pneumatic cell of the tympanic tegmen, a vascular conglomerate that continues in an osseous canal across the roof of the tympanic tegmen. The cells of the tegmen over the tympanic cavity remained in their greater part pneumatic, a minor portion showed cancellous content and infrequent adipose tissue.

The *tympanic cavity* remains, from the apex to the mastoid and including the few cells located in the latter in direct connection with the pneumatic cells. Pneumatization is "ideal" and maximal everywhere. Meyer stated that in certain regions of the cochlea pneumatic cells replaced the entire periosteal layer Within the richly pneumatized area great variety can be observed.

The large ossicles present, as against humans, a somewhat twisted form. This shows up in cross sections, as tripartite





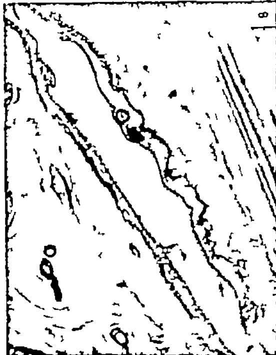


Fig 5 Scarce vessels at location of the fremla. ×32.
Fig 6 Secondary tympanic membrane exposed by lack of promontorial subloplasm. ×32

Fig 7 Elevation of lateral half of Corti papilla, tectorium retracted.
Fig 8. Vestibular aqueduct. Roughness of edge of bone underlying soft tissue wall of canal. ×60

fields (Fig. 4) produced by crescent-shaped tortuosities. The *stapes* shows slender contours compared to the more squat human figure

The *carotid canal* with its scalloping lamellae penetrated the cochlear shell to such an extent that the periosteal layer was completely repressed and replaced (Fig. 14).

Internal ear

The *cochlea* with three turns shows the human formation, i.e. flattened, as compared with the cochlea of the guinea pig. The structure of the capsular bone is similar to that seen in humans (Haag, 1933) Starck pointed to the very considerable differences in the sequence of ossification of the inner ear capsule in different mammals. Comparatively wide spaces filled by calcified blocks of cartilage delineate an enchondral layer Around endoglobular islands ample blue mantle formation was in progress.

Bast (1933) did not find a *fissula* in any of the laboratory animals from rat to monkeys, but he quoted related findings of Hyrtl (1836) in hyena and antelope. In the present material a vascular conglomerate of moderate size occupied the fissular site (Fig. 5)

Of practical significance are the conditions around the *subiculum* In rhesus it was reported as practically lacking (Kelemen, 1968, Figs. 5 6) leaving the niche of the round window opposite the tympanic membrane open to a direct approach and giving com- ively easy access to the cochlea. In be- n the niche is somewhat more closed with tip of the promontory bent inward (Fig. 6). The niche still remains wide open and offers access to the membrane of the round window in its entire extent. However as the tympanic membrane cannot be visualized by otoscopy at least not without some surgical intervention, this *per se* advantageous situation cannot be exploited to full extent—in marked contrast to rhesus. The plane of the membrane of the round window runs at right angles to the plane of the tympanic membrane even so, approach to the membrane of the round window after manipulation of the external canal, is easier than in humans, as the obstructing free tip of the subiculum is much shorter

A subiculum fissure was not observed contrary to Haag, who noted in the inner ear capsule of monkeys congenital fissures comparable to those in man.

Conspicuous *cartilage blocks* in the capsular bone could point to a young age for the individual (Starck, 1965).

The *organ of Corti* is characterized, as to general contour by a considerable elevation in its lateral half (Fig. 7) against the more even dome of the human organ. The tectorial membrane was mostly pulled back, foreshortened by shrinkage, and leaving behind the freed cellular hair

The *cochlear aqueduct* was, in most cases, entirely blocked by a fibrous-cellular mass. The *vestibular aqueduct* opened into a narrow lumen the soft tissue canal wall rested on a bony shelf with jagged edges and intensive osteoblastic activity (Fig. 8), concurring by this arrangement to the minimal permeability of the canal. The saccus, where present in the specimens, conformed to human conditions

PATHOLOGY

Among the 82 extant and examined ears 24 (29.5 %) showed pathological changes.

External ear

The most conspicuous finding has been a negative one absence of perforation of the tympanic membrane even with inflammatory processes in the middle ear

Middle ear

The *tegmen* or in general the pneumatized cortical portions of the entire temporal bone, carried the brunt of the morbid changes The cell masses of the cancellous content were transformed to sero-fibrinous secretions with or without suppurative infiltration in cellular patches (Fig. 9). Engorged central vessels, in the cells, with extravasation led to more extensive hematoma formation. In one instance blood filled a cell between the cochlea and the carotid canal. New bone formation in the cellular wall was not uncommon, and newly formed cartilage infrequently filled the lumen









Fig 9 Suppuration in cellular system of tegmen. ×12.
Fig 10 Tegmen cells, filled by granulation tissue and cartilage. ×3.

Fig 11 Subdural hematoma with intracranial penetration. ×60.
Fig 12 Suppuration in oval window ×44

in form of a chondroma (Fig. 10). In another case the cortical, underlying the dura, was penetrated amid abscess formation (Figs. 4, 12). Considerable thickening of the tympanic mucous membrane occurred but never led to polyp formation.

The endolymph and perilymph space of the vestibulum was seen filled with sero-fibrinous

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Fig 13 Vestibule: sero-fibrinous labyrinthitis. ×12.
Fig 14 Cochlea, sero fibrinous secretions and suppuration in all turns, suppuration in carotid canal. ×16.
Fig 15 Cochlea. Slight dilatation of Reissner's membrane in all turns. ×16.
Fig 16 Stapes: abnormality thick footplate, crura united and originating from center of footplate, suppuration in oval window ×16.

tory animals with infections in the nasopharynx (Nelson & Gowen, 1930).

Starck commented (1970) on the basis of a personal visit to locations inhabited by baboons, that occurrence of morbid manifestation can be very different accordin[illegible] separate regions.

ZUSAMMENFASSUNG

Unter 82 Pavian-Gehörorganen (in Serienschnitten untersucht) wiesen _4 (29,5%) histopathologische Veränderungen auf. Bemerkungen über die normale Struktur sollen die Interpretation der morbid-anatomischen Befunde erleichtern. Von experimentell-chirurgischer Konsequenz ist das Fehlen eines Subiculum, über der Nische des runden Fensters hängend, wodurch ein direkter Zugang zu den Schneckenräumen geboten wird. Dieser Gewinn wird durch den gewundenen Verlauf des äusseren Gehörganges verschmälert, ein Hindernis, das weggeschafft werden muss, um tieferliegende Gegenden operativ zugänglich zu machen.

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Acta Otolaryng 73 353–361 1972

A COMPARATIVE ANALYSIS OF MIDDLE–EAR FUNCTION IN NON–MAMMALIAN VERTEBRATES

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(Received March 17 1971)

Abstract. Mössbauer techniques were used to measure response patterns of the tympanic membrane and stapes footplate in five non-mammalian vertebrates. The response amplitude in microns was calculated for both structures at 100 dB S P.L. for tones between 62 Hz and 10.0 kHz. The response amplitude was constant for low frequencies while above 2 kHz it tended to rapidly roll-off reaching a rate of between 25–30 dB/octave. The results suggest that the limits of high frequency hearing in these species are related to the transmission characteristic of the middle-ear

There are many reports describing the middle-ear function of mammals. However as Grinnell (1969) has indicated, relatively little is known of the simple ossicular couplings of non-mammalian vertebrates. The advantage of the three-bone mammalian ear over the single bone columella of non mammalian vertebrates is an unresolved issue. Most investigators simply consider the columella middle-ear as both anatomically and functionally a relatively primitive system. The mammalian ear however is usually thought to be a higher achievement in the scheme of evolution. Tumarkin (1955 1968) and Webster (1966) have objected to this orthodox morphological approach and argue that any evolutionary consideration of ear anatomy must take into account its functional significance to the organism. These arguments are somewhat premature since a precise description of the peripheral transduction process comparing the *function of homologous organs in a variety* of species, has not yet appeared. One of several difficulties in such an analysis lies in determining what middle-ear function should be examined. A description of tympanic membrane and columella footplate motion, in absolute amplitude, for a constant S.P.L. is perhaps the most relevant function to be studied. Knowledge of these events would permit a critical evaluation of middle-ear processes between species and might provide evidence for a "hearing limitation" within given species.

The introduction of Mössbauer procedures (Hillman et al., 1964 Johnstone & Boyle, 1967 Johnstone et al., 1970· Taylor & Johnstone, 1970· Gilad et al., 1967) to the study of middle ear responses has resulted in a reappraisal of problems which, until now has not been feasible. These procedures are used in the present report to describe the transmission characteristics of the middle-ear mechanism in amphibian, reptilian, and avian species. A comparison is also made between these responses and similar ones in the mammalian ear

METHOD

Five species of non-mammalian vertebrates were studied. Two amphibian species were represented the toad *Bufo marinus* and frog

This research was supported with funds from the Australian Research Grants Commission and University research funds to Dr B. M. Johnstone.

Hyla dentata. These animals were immobilized with an intraperitoneal injection of allo-ferin (0.5 mg/kg) and a total of five ears in *B. marinus* and eight ears in *H. dentata* were tested. Reptilians were represented by the spotted gecko *Phyllurus milli* (five ears) and the dragon lizard *Amphibolurus reticulatus* (six ears). These subjects were anaesthetized with sodium pentobarbital (30 mg/kg). The tympanic membrane was surgically exposed by retracting the skin flap over the shallow external meatus. A bony shelf forming the posterior wall of the gecko's ear canal was also removed. The middle-ear was exposed with an opening in the lower jaw close to the posterior end of the mandible. Five ears of one avian species, the dove *Streptopelia risoria* were tested. Each dove was anaesthetized with a gaseous mixture of halothane and oxygen after the methods of Schwartzkopff & Bremond (1963) and Myers & Stettner (1969). The superficial tissue forming a shallow ear canal was removed to provide a clear exposure of the tympanic membrane. The avian's middle ear was also surgically ex[posed] with the posterior-dorsal approach de[scribed] by Wever & Bray (1936). The condi[tion] of each animal was checked during the experiment with the aid of a cardiac monitor.

Amplitude of the tympanic membrane and columella response was measured in a stereotyped manner. Test frequencies were selected between 62 Hz and 10 kHz. These frequencies usually progressed in octave intervals, but where necessary intermediate frequencies were used. The test tone intensity varied between 80 and 120 dB S.P.L. and was always adjusted to the lowest level that would permit an accurate determination of response amplitude.

The anaesthetized animal was secured to the operating table with plasticine. Additional plasticine was moulded into a dam surrounding the tympanic membrane and a Mössbauer source (Co^{57} imbedded in stainless steel) about 100 μm in diameter was fixed to the center of the drum in a position opposite the tip of the manubrium, with a spot of grease. A sound-input tube and microphone-input probe tube (Brüel and Kjaer 12.7 mm microphone and 1.0 mm probe) mounted in a thin plastic cap was positioned within a few mm of the drum. The probe tube was sealed to the tympanic ring by compacting plasticine around it. The top of the cap was clear and permitted counting of the 14 KeV gamma rays emitted by the source as well as visual inspection of the eardrum. Sound pressure at the drum surface was measured through the probe tube in dB re 0.0002 dynes/cm². The Mössbauer system has been described elsewhere (Johnstone & Boyle, 1967; Johnstone et al., 1970). In summary, radiation from the Co^{57} source was counted through a stainless steel absorber. A test tone was presented in the repetitive sequence of 7-sec on and 7-sec off and sound pressure was adjusted until the count rate, with sound present, was 10–60% greater than with sound absent. Counting usually continued for 3 min after which the percentage difference was transformed to velocity, corrected to velocity at 100 dB and then converted to absolute peak amplitude in microns. This procedure was repeated for each test tone.

The middle-ear of each species was opened to eliminate the resonant properties of the bulla cavity (Møller, 1965; Guinan & Peake, 1967). This was achieved in the amphibians by simply opening the jaw and exposing the eustachian tube. The middle ear of the bird and lizards was surgically exposed as previously described.

After the responses of the drum were measured the probe tube, plastic cap, and source were removed from the tympanic membrane. The animal was reoriented so that the columella footplate could be visualized in an operating microscope. The source was placed gently on the columella and nudged into position so that two criteria were met: first, there should be minimum tissue obstruction in a line from the source through the tympanic membrane to the counting tube and secondly, the source should be as close as possible to the colu-

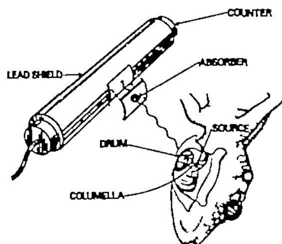


Fig. 1. The drawing shows the gecko middle ear exposed from the ventral side. The line from source to counter represents the path of the 14 KeV gamma rays. The sound probe was placed over the eardrum and the counter was oriented as close as possible to the ear (usually about 25 mm). The animal and counter are not drawn to scale.

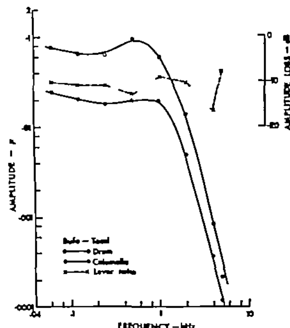


Fig. 2. Middle ear amplitude as a function of frequency for the toad *Bufo marinus* scaled to a constant sound level input of 100 dB S.P.L. The dotted line shows the theoretical amplitude of an air particle at 100 dB S.P.L.

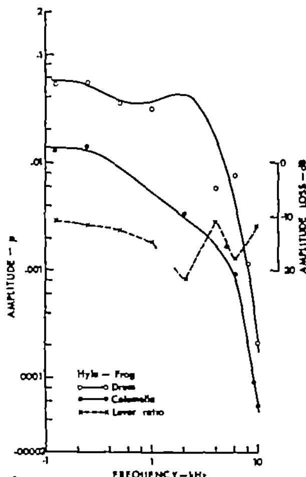
mella footplate (Fig. 1). The mucous membrane overlying the middle ear structures provided sufficient adhesion to secure the source to the columella. The probe tube and cap were repositioned over the ear drum and the procedures used to measure the response of the tympanum were repeated for the columella. Similar test frequencies were employed and typically the S.P.L. had to be increased by 10–20 dB to compensate for amplitude loss across the middle ear. All columella foot plate responses were converted to absolute amplitude in microns at a standard intensity of 100 dB S.P.L.

The present procedures measured only the simple piston-like movements of both the tympanic membrane and footplate. This was assured by always orienting the counter tube at right angles to the animal's ear drum. A slight misalignment between the direction of motion and the axis of measurement, due to middle-ear anatomy was frequently encountered when the footplate was examined. This misallignment rarely exceeded 20–30 and was well within the solid angle acceptance of the Mössbauer system.

Although it was possible to measure the tympanic membrane response in every ear tested, a correlated measure of drum and footplate amplitudes in the same ear was not always possible. However in each species studied, both responses were obtained in at least two or more ears. Although this sample may seem small, the functions obtained for subjects within a species were almost identical. At the end of a testing session the subject was given a lethal dose of anaesthetic and the general post mortem condition of the middle-ear was examined.

RESULTS

The tympanic membrane and footplate response for each ear in which both measures were obtained were averaged within a species and plotted together. Furthermore, the ear drum amplitude was divided into the footplate amplitude at each test frequency and the re-



3 Middle ear frequency response for the frog *dentata* (at 100 dB S.P.L.). The dotted line ows the theoretical amplitude of an air particle at 100 dB S.P.L.

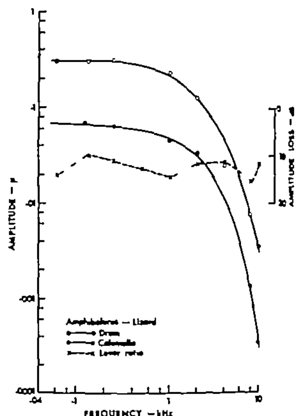


Fig 4 Middle ear frequency response for the lizard *Amphibolurus reticulatus* (at 100 dB S.P.L.) The dotted line shows the theoretical amplitude of an air particle at 100 dB S.P.L.

sulting inverse ratio was plotted along with the response curves for each species. This ratio described the amplitude reduction from drum to footplate response and was expressed as a dB amplitude loss. The theoretical amplitude of an air particle is indicated in each figure and provides a comparison for the mechanical responses of the middle ear This theoretical amplitude was calculated for 100 dB S.P.L. and would represent the ear drum motion for a perfectly matched ideal" system.

Fig. 2 shows the middle-ear transfer characteristics of the toad *B marinus*. The amplitude of both drum and footplate remained relatively constant from 62 Hz to 1 0 kHz. At higher frequencies the response of both structures rapidly rolled-off reaching a rate of about 25 dB/octave. The response amplitude of the tympanic membrane was always larger than the footplate and this relationship was consistent in all species. The inverse ratio function indicated an amplitude loss across the middle ear of approximately 12 dB. Fig. 3 illustrates the responses in the other amphibian, the frog *H dentata*. The drum response exhibited a relatively constant amplitude of about 0.05 μm between 125 Hz and 2.0 kHz. At higher frequencies the amplitude rolled-off at 28 dB/octave The footplate response showed two characteristics which were a roll-off of 6 dB/octave between 125 Hz and 4 0 kHz and then a sharp drop-off at about 28 dB/octave between 5 0 and 10.0 kHz. The amplitude loss across the middle-ear was rela-

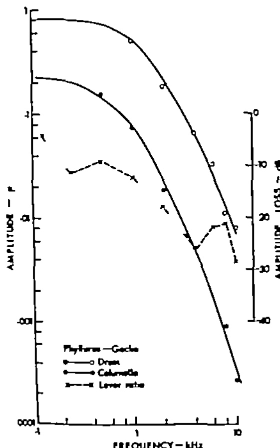


Fig 5 Middle ear frequency response for the lizard *Phyllurus milii* (at 100 dB S.P.L.). Note the frequency dependent lever ratio

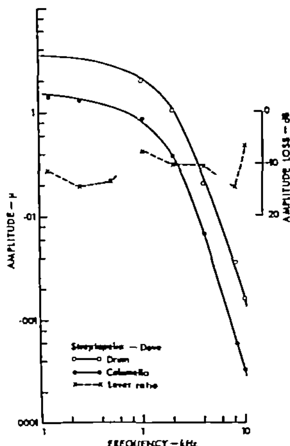


Fig 6 Middle ear frequency response for the dove *Streptopelia risoria* (at 100 dB S.P.L.). The dotted line shows the theoretical amplitude of an air particle at 100 dB S.P.L.

tively constant (13 dB) between 125 Hz and 1.0 kHz. At higher frequencies the function was variable, but seemed to represent a loss of about 15 dB

Post-mortem analysis of the amphibian ears revealed a massive mucous membrane surrounding the columella in *B marinus*. This tissue was continuous with the drum and bony walls of the middle ear and appeared ciliated. A similar though less massive tissue layer was observed overlying the middle ear structures of *H dentata*.

Fig. 4 shows the response patterns observed in the dragon lizard. Both drum and foot plate showed a constant amplitude of about 0.3 μm and 0.06 μm respectively between 62 Hz and 2.0 kHz. At higher frequencies the response rapidly rolled-off reaching a rate of 30 dB/octave at 10.0 kHz. An amplitude loss of 13 dB across the middle ear was relatively constant at all frequencies.

The responses of the spotted gecko (Fig. 5) were somewhat different. The amplitude of the drum and footplate were respectively 10 and 15 dB more sensitive at 100 Hz than in the dragon lizard. The amplitude of the drum response was relatively flat between 0.1 and 1.0 kHz while at higher frequencies it rolled-off at 20 dB/octave. The foot-plate response, however showed an amplitude reduction 6.0 dB/octave at the low frequencies and a much higher rate (28 dB/octave) as the frequency rose. Moreover whereas the inve

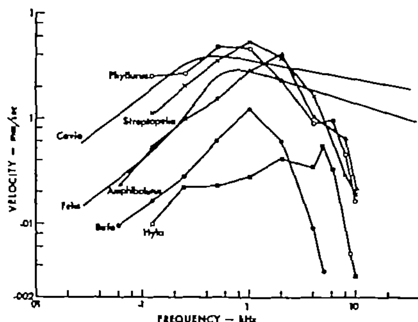


Fig 7 A comparison of reptiles, birds and mammals. The graphs are plotted as velocity/frequency response curves at 100 dB S.P.L. As the sound pressure is constant, the air particle velocity is constant, hence these graphs are in the input/output form usually associated with filters and electrical circuits. They show clearly the low frequency fall-off at about 6 dB/octave below approx. 500 Hz. The columella ears have a fall-off at about 14 dB/octave at 10 kHz compared with 1 to 4 dB/octave for the mammalian ears.

the dragon lizard was independent of frequency the gecko exhibited a frequency dependent loss varying between 5 dB at 200 Hz and 30 dB at 10.0 kHz.

Fig. 6 shows the responses in the barbary dove. The motion of the tympanic membrane was similar to that observed in the dragon lizard and at 10 kHz exhibited a roll-off of about 27 dB/octave. The footplate response ... the same general transmission characteristic but with a 10 dB amplitude loss at all frequencies.

The middle-ear anatomy in the lizard and avian species exhibited the delicate simplicity described by other authors (Gaudin, 1968 Wever 1965). A striking difference in these subjects when compared with the amphibian was the absence of massive membranous tissue surrounding the columella.

In all species studied the response of the tympanic membrane came closest to showing the theoretical maximum amplitude at the point of inflection between the low frequency characteristic and the high frequency roll-off. The gecko was closest coming within 2 dB and the amphibian farthest at 20 dB

The footplate responses of all species are summarized in Fig. 7 and have been replotted in terms of velocity Since these figures are at constant S.P.L. i.e. constant air particle velocity the graph represents relative transmission and is similar to a standard filter response curve. The velocity of stapes movement in the guinea pig (Cavia) and cat (Felis) are also presented for comparison (Taylor & Johnstone, 1970). Fig. 7 clearly shows that the frog and toad responses were much less sensitive than those observed in the other animals. When cat, guinea pig, dove and lizards were considered the velocity of stapes and footplate response at 1 0 kHz had a range between species of only 5 6 dB. At frequencies above 2.0 kHz, however the mammalian stapes response rolled-off at about 1 dB (guinea pig) and 3.7 dB (cat) per octave whereas the velocity in the non-mammalians rolled off at about 14 dB/octave.

DISCUSSION

The middle-ear is generally considered as a transformer matching the impedance of air to that of the cochlear fluids. The middle-ear suffers a transmission loss since it contains real elements which have mass, stiffness and friction. This transmission loss is frequency

dependent and hence varies the characteristics of the sound energy reaching the cochlea. The final "hearing" capacity of an organism is, of course, bounded by these characteristics.

The present results suggest that the middle ear of the non-mammalian vertebrate is operating as a piston containing elements with mass, friction and stiffness. Wever & Lawrence (1954 p. 389) and more recently Møller (1965) have described the contribution of these components to the middle-ear transmission function. Stiffness determines the low frequency response of the system, mass operates at high frequencies, and frictional resistance limits the response at some intermediary frequency A description of these elements in terms of anatomical structures is a matter of debate. However extracting from Wever & Lawrence s interpretation for the mammalian middle-ear (1954 p. 389) to the simpler columella ear would suggest the following. The total mass of the system is derived from a combination of the tympanic membrane, the structure of the columella and extra columella, the general suspensory system consisting of supporting ligaments, the middle-ear muscles, and ligaments supporting the footplate in the oval window and finally the fluids of the cochlea. Stiffness is derived mainly from the drum membranes, the suspensory system and the relative ease with which the cochlear elements are free to move. The frictional component results from the dissipation *of energy due to the movement of* elements in a vibrating system.

The characteristic response curves for each species clearly delineate these components. The relatively constant amplitude of low frequency responses (below 1 0–2.0 kHz) represent a transmission characteristic determined by stiffness. The rapid decline in response amplitude at higher frequencies was typical of a transmission curve determined by the mass of the system. The amplitude of responses at the inflection point between 0.5 and 2.0 kHz can be accounted for by the frictional losses of the middle-ear Unfortunately the precise biophysics of these analogical elements are not known at present.

The differences between tympanic membrane (as measured at the manubrial tip) and footplate response amplitude (the inverse ratio) indicate the classic lever ratio across the middle ear Interestingly enough, the lever ratios in the present species were considerably greater than those reported for mammalian species. Guinan & Peake (1967) note a frequency dependent level ratio only above 7 0 kHz in the cat. Below this frequency however the ratio is relatively constant at 2.0. Wever & Lawrence (1954 p. 107) report an overall ratio in cat of about 2.5 while von Békésy (1960, p. 102) describes a generally accepted lever ratio of 1.3 for man. It would appear that the lever ratio in both man and cat is significantly smaller than those presently observed in the non-mammalian vertebrates. In these species, lever ratios between 3.1 and 5 0 were not uncommon while the gecko at 10.0 kHz even showed a ratio of 13 0. Moreover with exception of the gecko, the lever ratios seemed to be independent of frequency at least within the range tested. It should also be recognised that the present methods were only sensitive to pistonlike movements of the drum and footplate. Other more complex movements, if they exist, might contribute significantly to the large lever ratios. Our observations of the footplate during gentle probing of the ear drum however *only suggest a simple in-out displacement* of the footplate. The high lever ratios indicate that the force exerted on the fluids of the cochlea may be relatively large and what function this serves for the process of "hearing" in these species is uncertain. A more complete analysis must await phase data and footplate and eardrum area ratios.

In general, the important feature of the present results lie in their contribution to the comparative analysis of "hearing" It is accepted that the "hearing" capability of non-mammalian vertebrates as measured by the

a.c. cochlear potential (Wever & Bray 1936 Hepp-Reymond & Palin, 1968 Strother 1959 1962) evoked response audiometry (Loftus-Hills & Johnstone, 1970) or behavioral conditioning (Schwartzkopff 1963 Konishi, 1970 Heise 1953 Strother 1962) exhibit a high frequency capacity which rarely exceeds 10.0 kHz. Sharply contrasting this is the phenomenally well developed "hearing" ability in terms of both absolute sensitivity and high frequency sound detection, of all mammalian species. Masterton et al. (1969) have clearly demonstrated the high frequency capability of mammals and have summarized the relevant literature describing this phenomenon. They suggest that the anatomical structures of the middle-ear are most likely to account for the high frequency differences between mammals and non-mammals. The present results when compared with the middle ear characteristics of cat and guinea pig provide empirical support for this suggestion. About 10 0 kHz there is little functional energy (expressed in either velocity or amplitude terms) communicated to the cochlear receptor of the non-mammalian ear On the basis of the species presently reported it would that the differences in high frequency ...ring between animals having columellas or ossicles may in part be accounted for by the middle-ear transmission function. This interpretation should be treated with some caution however since Wever & Lawrence (1954 p. 78) have shown that the sensitivity of a.c. cochlear potentials recorded from the round window has the same general shape across frequencies when sound is transmitted via the normal middle-ear path or directly to the oval window It may be that the physical property of the cochlear fluids load the stapes foot plate in a way that contributes significantly to the middle ear transmission characteristic. Unfortunately middle-ear function has not adequately been studied with the fluids of the cochlea removed.

The absolute sensitivity of the ossicles and columella suggests that they function equally well, at least below 3 0 kHz. Any superior "hearing" sensitivity of mammals in this region probably reflects the greater sophistication of the cochlear organ and a more advanced analytical capability in the nervous system.

Finally it is interesting that a "columella" ear has stapes responses within 6 dB of a 3 bone ossicular chain "mammalian" ear over the range of "communication" frequencies below 3 kHz. This suggests that a "columella-like" prosthetic, frequently used in middle ear reconstruction, is about as efficient a procedure that can be achieved. A more complicated "ossicle-like" reconstruction while not only mechanically difficult to install would not appreciably add to the restored hearing.

ACKNOWLEDGMENTS

The authors wish to thank the Department of Zoology University of Western Australia the C.S.I.R.O. Avian Research Station, and Mr J Loftus-Hills, Department of Zoology Melbourne University for providing the subjects. The assistance of Miss H. Rae is also gratefully acknowledged.

ZUSAMMENFASSUNG

Mössbauer Methoden wurden verwendet um die Reaktionsweisen der tympanischen Membrane und der Fussplatte des Steigbügels in fünf nicht säugetierartigen Wirbeltieren zu messen. Der Reaktionsumfang in Mikronen wurde für beide Strukturen bei 100 dB S.P.L. in einem Tonbereich von 62 Hz bis 10.0 kHz berechnet. Die Reaktionsamplitude war konstant für niedere Frequenzen, während sie bei über kHz zu einem schnellen "roll-off" neigte indem ein Bereich (Verhältnis) von 25–30 dB/Octave erreicht wurde. Die Resultate zeigen, dass die Limiten des hohe Frequenzen Hörens in diesen Exemplaren mit den Übertragungseigenschaften des Mittelohres in Verbindung stehen.

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Acta Otolaryng 73 362–373 1972

MUSCLE ACTIVATION FOR LABIAL SPEECH GESTURES

An Electromyographic and Acoustic Study of Normal Speakers

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(Received June 30 1971)

Abstract The present study demonstrates the existence of several electromyographic and acoustic characteristics common to a normal-speaking group during labial articulation Prespeech, background and articulatory activity have been identified and distinguished from each other The muscles are recruited in two functionally antagonistic groups with reciprocal innervation. There is clear evidence that the EMG activity is context-dependent. Vowel duration varied systematically as a function of both vowel identity and consonantal environment. The duration of labial closure varied in a complementary fashion with the duration of the vowel segment. The dependence of vowel duration on the voicing or voicelessness of the following consonant was correlated th a difference in the timing of the EMG activity lated with the labial closure gestures. This timing also found to depend significantly on the identity f the vowel. To account for these latter results a model of timing is proposed according to which the moment of gesture initiation (= onset of EMG activity) is a function of the articulatory and acoustic features of the gesture to be executed, such as its spatial extent and duration. Although more research is necessary on the neurophysiological correlates of such a hypothetical mechanism, the present findings for normal-speakers may provide a preliminary baseline for clinical applications and the further study of articulatory disorders.

The principal aim of the present investigation has been to extend a previous EMG study on labial articulation (Leanderson et al., 1971) which involved only one subject. We have *attempted to ascertain whether the patterns of* EMG activity which were found to be reproducible intra-individually appear also in other normal-speaking subjects and whether common EMG characteristics can be established for a whole group of subjects. Such information might then serve as a basis for clinical applications involving comparisons of normal and pathological conditions of speech.

Few studies have been published on the *articulatory function of the labial musculature* with more than one subject (MacNeilage, 1963 Harris et al., 1965 Hirose et al., 1969 Sfs, 1970) Most of the topics analysed and presented in the current study have not been investigated *electromyographically before.* They have, however been dealt with in other kinds of speech research and discussed in the literature prespeech and background activity ("Neutral position" Chomsky & Halle, 1968) muscle recruitment underlying gesture execution (Bernstein, 1967) context dependence in the realization of phonetic segments (Öhman, 1966). In the present study particular interest is devoted to the aspects of *timing of articulatory activity* The data are related to some current phonetic models of temporal mechanisms of speech production (Kozhevnikov & Chistovich, 1965 Lenneberg, 1967 Ohala, 1970 Lehiste, 1970).

METHODS

For the present study 10 adult subjects were chosen 7 female and 3 male, with acoustically

This work was supported by the Swedish Medical Research Council (Project No. B71-61P 3213-01) and the National Institutes of Health (Research Grant NS 04003-09).

and phonetically normal articulation (5 subjects were speech therapists) All subjects were examined under similar and carefully controlled experimental conditions. The procedures and the recording technique have been described in detail previously (Leanderson et al., 1971) All observations were made on Swedish nonsense words of /səpa dV ₁CV₋₁ dare/ structure. The intervocalic consonants were [p], [b] or [h], coarticulated with [i], [y] or [ʉ] so as to form all possible combinations. The test words, which had the so-called grave accent pattern, were presented to the subjects on randomized cards at a certain tempo Each subject produced three repetitions of each test item. The tape-recorded materials were processed on an ink writer at 1/10th of the recording speed. No integration was used except for that involved in direct recording. In order to obtain better resolution of the recorded signals and facilitate more accurate observations, the paper speed was increased from 10 mm/sec as used earlier to 25 mm/sec. The timing of the following acoustic and electromyographic events was measured

Initiation of the first vowel segment (V_1), i.e. release of [d] On set of lip-closing activity as revealed by EMG Beginning of consonant segment, i.e. end of voicing when C – [p] and beginning of closure when C – [b]. Onset of lip-opening activity for [p] and [b] as displayed electromyographically Initiation of second vowel segment (V_2) or release of [p] and [b].

All temporal measurements were made by the same investigator for whom repeated controls showed good consistency the means of each individual subject falling within a range of 1 mm, i.e. 4 msec. The estimation of time locations of acoustic boundaries and electromyographic events inevitably depends to some extent on recording gain and signal/noise ratio Care was taken, however to ensure optimal conditions during the accumulation of data. As shown in Table I the S.E.M. (standard errors of means) associated with the measurement of EMG activity are smaller in general than those for the acoustic data. Since the latter are on a par with what is normally accepted in temporal acoustic phonetic measurements, the smaller S.E.M. appear to indicate that the estimation of EMG events was made with at least comparable reliability

RESULTS

Background activity

As exemplified in Fig. 1 each normal-talker appeared to have a characteristic EMG pattern, comprising background and articulatory activity the reproducibility of this pattern was good for the same utterance in the same subject. This result applies to all subjects. Most of the subjects could relax between the utterances. Some had a moderate resting activity Before the different utterances, many of the subjects assumed a preparatory speech posture of the lips. This speech initiation or prespeech activity was generally localized to M. lev lab and M dep. lab. and showed a durational variability from several hundred msec to a normal anticipation time for articulatory activity associated with the first phoneme of the utterance involving the lips. In some cases our data showed that a later onset of activity was associated with more prespeech activity possibly indicating a faster rate of articulator movement. The amount of background activity during the different utterances was fairly constant in each individual but varied considerably between subjects.

Muscle recruitment

Against this inter-individually variable tonic background pattern there appeared a phasic type of activity This occurred in all the muscles studied. The phasic activity was associated with the rapid articulatory lip movements and was systematically related to the acoustic segments recorded simultaneously For the rounding gestures of [y] and [ʉ] and the implosion phase of [p] and [b], M orb oris sup and M orb. oris inf were active together with M dep. ang. oris and M mentalis. For the lip-opening movement of [p]- and [b]-release and the lip-spreading gesture of [i], activity was found in M. lev lab. and M dep. lab On the basis of this well-coordinated articulatory activity the

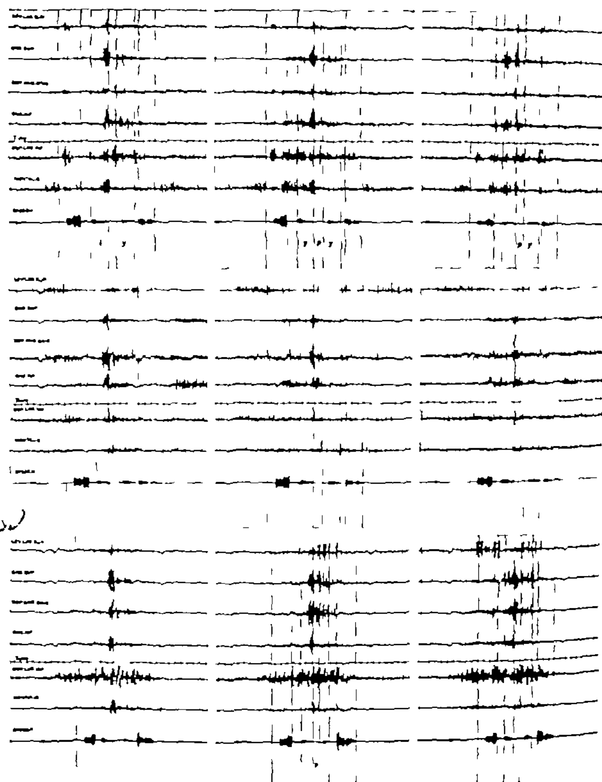


Fig. 1. Polygraphic recordings of speech and EMG activity in six facial muscles from three different normal speakers during three different V_1CV_2 (vowel–consonant–vowel) utterances. Top row: Subject 1, middle row: Subject 2, bottom row: Subject 3. Time scale 100 msec.

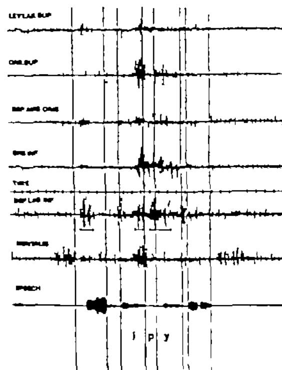


Fig. 2. EMG recording of an [ipy] utterance showing two types of articulatory activity in M. dep. lab., (*a*) lip-opening and -spreading activity for [i] and [p]-release, marked with continuous lines below the trace; (*b*) lip-rounding and -closing activity for [y] and [p]-implosion, marked with dotted lines. Time scale 100 msec.

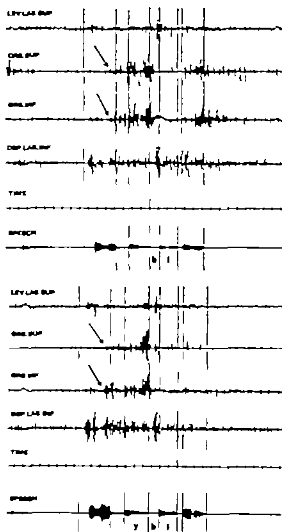


Fig. 3. EMG activity for the production of [u] as compared with [y]. The initial activity in M. orb. sup. et inf., similar for both vowels (arrows), increases at the [u] segment boundary to a sudden burst, shaped like the following [b]-implosion activity. Time scale 100 msec.

muscles could thus be divided into two functionally antagonistic groups, the composition of which was principally the same for all the normal-talkers of the present study. The amount of activity in the different muscles within the same muscle group varied between individuals. One subject had strong and equal activity in M. orb. oris sup. and M. orb. oris inf. for [y], while another had activity predominantly in M. orb. oris inf., in a third subject the most active muscles for this phoneme were M. dep. ang. oris and M. orb. oris sup. In addition to its primary function, a particular muscle could be activated for other gesture movements. As shown in Fig. 2, M. dep. lab. may have a lip-spreading function in the production of the initial spread segment of the carrier phrase and the following [i]. For the release of the stop consonant it opens the mouth by pulling down the lower lip. Its function in speech posture has been mentioned already. This muscle is also activated, however, for the [p]-implosion and [y] in co-contraction with M. orb. oris inf., the lip-rounder/closer.

In 8 of the subjects the vowel /u/ was produced by two types of lip-rounding activity in the same muscle. In M. orb. oris (Fig. 3) an initial activity of low intensity starting about 200 msec before the acoustic segment, as is the case for

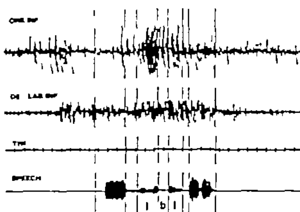


Fig 4 Reciprocal inhibition of the prespeech EMG activity in M orb. inf. Time scale 100 msec.

[y] suddenly changes to a stronger activity starting within or at the boundary of the acoustic segment. An informal auditory analysis revealed a characteristic diphthongal pronunciation [uβ] of the /u/ vowel. In the realizations of the /y/ phoneme the bilabial [β]-glide was never heard. Nor was it expected since the diphthongization of the [y] when present normally consists in the insertion of a non-labial, palatal [yj] (cf Hammarström & Norman, 1957) We interpret these auditory observations to be compatible with the above-mentioned EMG findings.

...cal inhibition

In spite of the co-contracting antagonistic activity which appeared in 4 of the subjects in the lip-opening muscles during [p]-implosion as well as in the lip-closers during [p]-release all subjects manifested clear signs of reciprocal inhibition (Fig. 1) In the traces from Subject 1 the activity in M orb oris sup disappears or decreases during [p]-release In Subject 2 the activity in M dep lab is inhibited during [y], and in Subject 3 a period of inhibition is visible in M lev lab during [u]. In Fig. 4 there is an obvious reciprocal inhibition of the prespeech lip-positioning activity in M orb. oris inf while an adequate lip-lowering activity appears in M dep. lab.

Context dependence

In agreement with previous findings from one talker (Leanderson et al. 1971), the present normal-speaking group had the characteristic in common that the degree of activation of a certain muscle for a certain phoneme depended upon the activity for the preceding phoneme. If phonemic segments were the same or similar (see the V_1CV_2 combination [yhy] in Fig. 5) the activity decreased before or during the second vowel. Note that this decrease occurs as early as before the first vowel of [ihi] (Fig. 5) because of the spread segment of the preceding carrier phrase.

The activity for [i] in M lev lab and M. dep lab. can be seen to be delayed through inhibition in [upi], where it starts about 50 msec before the onset of the [i]-segment, as compared with [ubi], where it starts even during [u] and about 200 msec before [i] is initiated (Fig. 6)

The [p]-implosion activity was generally stronger after the spread [i] than after the rounded [y] (Fig. 7)

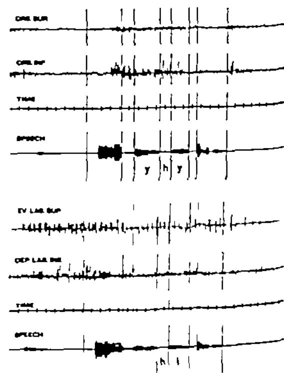


Fig 5 Decrease of EMG activity in M orb. oris sup. et inf. and M. lev et dep. lab. for the second vowel in V_1CV_2 utterances where $V_1 = V_2$. As the latter muscle group is active for the preceding contextual frame, the activity decreases already for the first [] Time scale 100 msec.

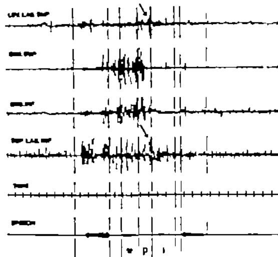
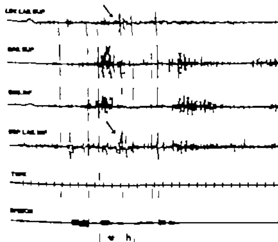



Fig. 6. Anticipation time of the EMG activity in M. lev. et dep. lab. for [i] when this vowel is preceded by a non-labial and a labial consonant respectively (arrows). Time scale 100 msec.

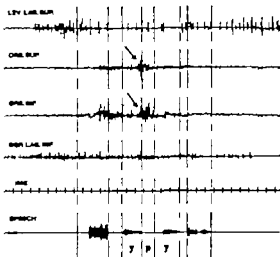
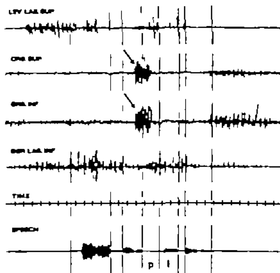



Fig. 7. Implosion activity for [p] in different vocalic contexts. The EMG activity in M. orb. oris sup. et inf. starts earlier and is stronger after [i] than after [y] (arrows). Time scale 100 msec.

Durational characteristics

Average data on acoustic segment durations and onsets of EMG activity are presented in Figs. 8–10 and Table I. Each point in the figures represents the mean value of a total of 90 measurements. The figures are accounted for as follows. Exploratory plotting indicated that the temporal events within V_1C sequences (C = [p] or [b]) were independent of the identity of V_2. Conversely the temporal events within CV_2 were independent of V_1. It was therefore decided to pool these transconsonantal vowels, which are indicated by the braces in Figs. 8–10. The averages are consequently based on 90 measurements (3 repetitions × 3 vowel contexts × 10 subjects). The figures prompt the following observations.

I. Acoustically vowels seem to be longer before [b] than [p] (Fig. 8). Note that "vowel duration" is defined by non-identical criteria in the case of [p] and [b]. The end of the vowel segment is taken to be the termination of voicing for [p] and the beginning of closure for [b]. From the

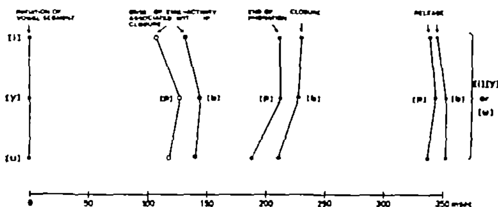


Fig. 8. Time locations of electromyographic and acoustic events in VCV utterances with the vowels [i], [y], and [ʉ] and the consonants [p] and [b] in all possible combinations. Each point represents a mean of nine observations from each of 10 subjects. The time reference is the release of [d] before V_1. The consonantal events are measured only in relation to V_1.

acoustic record it is not possible to determine when the lips come together for [p]. The labial closure may not be synchronous with the end of vocal cord vibration (cf. Slis, 1968; Karlsson & Nord, 1970).

II. For [i] and [y] the segment durations are approximately the same in a given consonantal context. On the other hand [ʉ] comes out significantly shorter in both cases (comparing the mean for [ʉ] with that for either [i] or [y] ~ <0.01 before [b], $p<0.05$ before [p]).

III. The timing of the [p]- and [b]-releases ... less than the beginnings of these consonant segments. Thus, releases all occur within a range of approximately 20 msec, whereas the terminations of voicing for [p] and the [b]-closures are spread over an interval of about 40 msec (Fig. 8).

IV. The EMG activity for lip closure appears earlier for [p] than for [b] measured from the initiation of V_1, i.e. the release of [d] preceding V_1 ($p<0.01$ when V_1 = [i] or [y], $p<0.05$ when [ʉ]). The plotted onsets of EMG activity associated with the lip-closing gesture refer to M. orb. oris sup. and M. orb. oris inf. (Fig. 8).

V. The interval between EMG onset and the acoustic event indicating the beginning of the consonant segment is roughly independent of the consonant (Fig. 9).

VI. On the other hand, it is dependent on the vowel preceding the consonant. The interval decreases in the series [i], [y] and [ʉ] (comparing

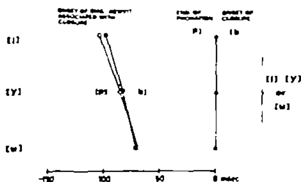


Fig. 9. Onset of EMG activity associated with lip closure related to the end of voicing for [p] and the beginning of closure for [b].

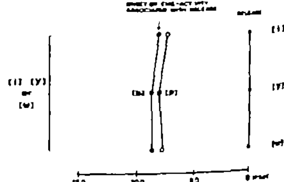


Fig. 10. Onset of EMG activity associated with release of [p] and [b]. The measurements are made in relation to V_2.

Table I. *Measurements in msec of acoustic and electromyographic events during V_1CV_1 utterances in which the vowels are either* [i], [y] *or* [ʉ] *and the consonants* [p] *or* [b]

The data represent averages based on ten individual means consisting of nine observations each

	[i]	[y]	[ʉ]
[p]			
Vowel (V_1) length	211±12.6	212±12.4	188±11.8
Onset EMG (implosion)—offset V	104±2.8	85±3.3	70±3.8
Onset V_1—onset EMG (implosion)	106±10.5	126±10.4	117±10.0
Consonant (C) length	128±7.2	131±5.2	149±7.6
Onset EMG (release)—onset (acoustic) release	74±4.9	81±3.2	78±5.2
[b]			
Vowel (V_1) length	229±11.0	227±10.7	211±9.9
Onset EMG (implosion)—offset V	98±3.8	83±3.9	71±3.7
Onset V_1—onset EMG (implosion)	130±9.6	146±10.9	142±9.7
Consonant (C) length	115±5.7	125±5.0	141±6.1
Onset EMG (release)—onset (acoustic) release	82±5.2	88±6.0	87±6.4

all means, $p<0.001$ when C = [p], $p<0.01$ when C = [b])

VII From Fig. 8 it appears that the [p]-segment has a slightly longer duration than the [b]-segment. Again, note the different criteria of segmentation.

VIII. More significantly there seems to be a compensatory balance between vowel and consonant durations in that both [p] and [b] are longer after [ʉ], the shortest of the three vowels (comparing the means for [i] and [ʉ], $p<0.05$ when C = [p], $p<0.01$ when C = [b]). As a consequence of this compensation, the releases span a narrower durational range than the vowel offsets (Fig. 8)

IX. The first traces of EMG activity for the [p]- and [b]-releases lead these events by about similar intervals (Fig. 10) The plotted onsets of EMG activity associated with the lip-opening gesture refer to M lev lab. and M dep lab

DISCUSSION

The present study confirms that previous observations on one subject (Leanderson et al. 1971) are valid for other normal-speakers as functional principles for lip muscle activity during speech production.

Prespeech activity

Our findings are in agreement with those of Fritzell (1969) who observed great variability in the timing of the velar levator activity in anticipation of the test utterance. Björk (1961) found that the very first closing movement of the soft palate for the beginning of speech started earlier and had a more variable onset time than within the test sentence. MacNeilage et al. (1970) found that the initiation of jaw movement from any prespeech to an initial target position began at various points in time. Our results are compatible with these observations.

Individual muscles in different speech gestures

Writing on a blackboard or on a small piece of paper demands similar character gestures but these are produced by completely different sets of muscles (Bernstein, 1967 as quoted by Ohala & Hirose, 1969). These investigators suggest that the speech apparatus might be described as a functionally output-oriented system in which the gesture predominates over the particular muscle, which follows the dictate of the gesture. In view of the listener-oriented function of speech communication (Jakobson et al., 1952) and the existence of individual differences in vocal tract anatomy it is not surprising that muscle activation and muscle group composition should display some inter individual variation with respect to the common characteristics.

Controlled articulatory movements

In several subjects, obvious signs of reciprocal inhibition as well as co-contraction of the antagonists could be observed in the EMG of the same utterance. The muscle group primarily responsible for the direction of the movement showed a

stronger activity however. This kind of activation might result in articulatory movements described by Stetson (1951) as controlled movements. Paillard (1960) denying the classic dichotomy between agonist and antagonist muscles, concluded that the cooperative action of a number of muscles is necessary for the production of skilled movements.

Context dependence

Our data agree with previous observations on labial coarticulation (e.g. Fujimura, 1961 Daniloff & Moll, 1968 MacNeilage & DeClerk, 1969). Thus, when the consonant in the V_1CV_2 combinations was the non-labial [h] there was always an obvious coarticulation between the second vowel and the consonant, the activity for V_2 starting well before the end of the acoustic segment of V_1 (Fig. 6). In combination with the labial [p] or [b] the inhibitory or dominant influence of the consonants did not permit the detection of similar anticipation phenomena in the EMG records. In fact, from the timing point of view the onset of activity for V_2 seemed to be more or less independent of the context.

Agreement obtains with the results of Lubker ... al. (1970) who found context-dependent variability for palatal levator activity. They suggest that this "activity is dependent upon where the palate is, and where it must go to prevent excessive nasal coupling".

Our data have shown, furthermore, that there is a great deal of systematic variability with respect to the temporal location of acoustic segment boundaries and related EMG events.

Vowel duration and articulatory distance

We shall examine our results on vowel duration in relation to a classical phonetic hypothesis, formulated by e.g. Jespersen (1926) and investigated by for instance, Fischer Jorgensen (1964). It states that a longer articulatory distance takes longer time. We shall claim that the data presented in Fig. 9 can be explained partly with reference to this hypothesis, partly in terms of a model of speech timing. Fig. 9 shows that the duration of the closing gesture, or more precisely the interval between the onset of EMG activity for [p]- or [b]-closure and the acoustic boundary representing the end of the acoustic vowel segment, is longest for V_1 = [i] shortest for V_1 = [ʉ] and intermediate for V_1 = [y]. Here it is helpful to recall the preceding remarks to the effect that these vowels are pronounced as diphthongs by many Swedish speakers. In narrow phonetic transcription they can be written [ij], [yj] and [uβ] (Hammarström & Norman, 1957). Compare also the comments above (Fig. 3) on the EMG activity for the production of [ʉ]. The labial diphthongal gesture characteristic of [ʉ], which it does not share with [y], has led some investigators to speak of inrounding for [ʉ] as compared to outrounding for [y] (Lyttkens & Wulff 1885). The inrounding gesture for [ʉ] here denoted by [β] approximates the closure position for [b] whereas outrounding as in [y] implies a different gesture. Frontal photographs of the lip openings associated with sustained productions of these vowels often reveal significant differences in area. Lindblom & Sundberg (1971) report the following average figures opening areas [i] = 183 mm² [y] = 30 mm² [ʉ] = 16 mm² (The subject on whom ... measurements were made happens to ha... ticipated in the present study too). These ... can be taken as a rough indication of distances that the lips must be moved from preceding vowel position for labial closure to obtained. Provided that the lip-closing ... ments are initiated at a given point in time du... V_1 and that they proceed at the same rate, ... would consequently expect the closing gest... initiated during [ʉ] to be shortest, that ... [i] to be longest and that during [y] to be ... mediate in length. This expectation is ... by the data of Fig. 9 and it seems justi... hypothesize that articulatory distance must ... invoked to explain these durational facts. H... ever the duration of V_1 is a function not only ... the duration of the labial closing gesture ... of the point in time where this gesture is ini... There appear to be two extreme timing strateg... according to which this gesture initiation ...

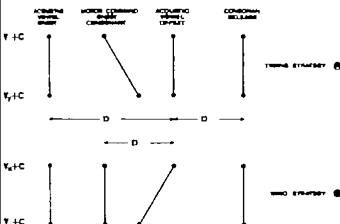


Fig 11 Idealized representation of two models of speech timing. D_V – duration of acoustic vowel segment, D_C – duration of consonant closure, D_T – duration of articulatory gesture. Model A is output-oriented, i.e. it produces vowel segments of identical duration in spite of the variation in D_T between V C and V_yC. The closure command for C is thus assumed to occur at a time that depends on when the goal of the gesture should be reached. Unless motor commands are thus timed with reference to their future articulatory and acoustic consequences, the alternative situation in B is obtained. In the text, model A is described as a predictive, *pre-planning strategy* whereas B is termed a *chain strategy*. As indicated by the present study observations fall somewhere between these two extremes.

be controlled (Fig. 11). Suppose that the goal set for the timing of the phonetic events of the V_1CV_2 sequence is to achieve an invariant acoustic duration of V_1 that is independent of the duration of the consonant closing gesture, D_T. To achieve this goal the control mechanisms must operate according to strategy A, that is, the timing of the motor command for the consonant gesture is adjusted according to the duration of the consonant gesture. With respect to the acoustic result this can be said to represent a predictive or *pre-planning strategy*. It is also possible to go to the other extreme by assuming that the timing is monitored in a fashion independent of the duration of the consonant closing gesture. This strategy is also shown schematically in Fig. 11 (strategy B). In this case the closing gesture is initiated after a fixed time has elapsed from the beginning of V_1. Consequently variations in the duration of V_1 will arise as a result of variations of closing gesture duration. This strategy is context independent at the level of motor command initiation. It might be described as a *chain strategy* that is, a blind process in which successive gestures are linked to each other without anticipation of factors such as the spatial extent of articulatory gestures. Fig. 8 demonstrates that the onset of EMG activity associated with lip closure occurs later during [y] and [u] than during [i]. This circumstance appears to exemplify timing strategy A, which seems to have been implemented with almost complete success in the case of [y] whose acoustic duration is comparable to that of [i]. During [u] on the other hand the initiation of the consonant closing gesture is similarly but not sufficiently delayed since acoustically [u] does in fact come out significantly shorter than [i] and [y]. The present data thus seem to indicate that timing of gesture initiation is adjusted with respect to the spatial extent of a given gesture but that this adjustment may be incomplete. The results are compatible with both a pre planning and a chain strategy (Kozhevnikov & Chistovich, 1965 Lenneberg, 1967 Ohala, 1970).

It is natural that we should find evidence of both mechanisms. During speech development and language acquisition an adult norm is imposed upon the child. This norm is auditorily defined and is interpreted by the child to indicate that under certain contextual circumstances it is normal for sound segments to

Acta Otolaryng 73 374–378 1972

PSEUDOMONAL GRANULOMATOUS EXTERNAL OTITIS

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(Received June 7 1971)

Abstract Five patients all in their seventh or eighth decade, suffering from external otitis due to *Pseudomonas aeruginosa* are described, four men and one woman. Concomitant diabetes was observed in two. Four patients recovered following systemic antibiotic therapy and limited local excision, and one died in spite of a similar treatment schedule His cardiovascular system showed few signs of aging and he had no diabetes. The invasive nature of this entity is stressed. Its causative background appears enigmatic.

Pseudomonas aeruginosa is a gram-negative, rod-shaped organism, strictly aerobic, which ...es a blue or green water soluble pig- ... It is widely distributed in nature, com- ...y found in water and soil, and sometimes in the human intestinal canal and on normal skin (Wilson & Miles, 1964). It is absent from the normal aural flora but may be associated with local suppuration in the external or middle ear as well as in the nose The organism was thought to flourish mainly as a secondary invader but recently it has been described as a primary pathogenic agent.

Pseudomonas aeruginosa is usually of low virulence, but it may acquire pathogenic features, especially in a tropical, humid climatic environment. The infection may be acquired in a hospital following surgery severe burns (Chandler 1968) or treatment by broad-spectrum antibiotics, corticosteroids and antimetabolites (Williams et al. 1960) Destructive lesions may appear on the skin, called ecthyma gangerosum. Infants, especially premature or newborn, are prone to develop severe systemic infection.

The pathological picture is characterised by vasculitis of venules and arterioles, associated with haemorrhages, thrombo-embolic phenomena and tissue necrosis.

If uncontrolled and not satisfactorily drained, infection of the external auditory meatus may spread to produce necrotising chondritis and osteomyelitis of the temporal bone. The inflammatory process often traverses the intercartilaginous dehiscences of the external meatal floor and penetrates the parotid loge the temporomandibular joint and the soft tissues of the neck. The most important diagnostic sign is persistent granulation tissue on the floor of the external auditory canal, near the junction of the cartilaginous and the bony portions. This finding requires immediate evaluation including culture studies, biopsy search for diabetes, X-ray of the mastoids and the skull. If the diagnosis of pseudomonal external otitis is established, early surgical debridement becomes mandatory as well as massive therapy with appropriate antibiotic agents (Chandler 1968).

Previous clinical experience

Meltzer & Kelemen (1959) were the first to describe the histological findings in osteomyelitis of the temporal bone zygoma and mandible caused by *Pseudomonas aeruginosa*.

Chandler described in 1968 a series of 13 cases of severe inflammation of the external auditory meatus, caused by *P aeruginosa* and affecting usually elderly diabetics. The disease was characterised by invasion of the aural cartilage and bone, resulting in paralysis of the facial nerve as well as the last four cranial nerves. Six of the patients died. Several factors could play a role in the fulminating course of the disease, an unusually high virulence of the causative agent, pre-existing damage to blood vessels due to diabetes and old age, and acquired resistance to standard antibiotic agents. The term malignant" was appended to this disease.

Morgenstern & Seung (1971) published a detailed histopathological description of the temporal bone of a 78-year-old diabetic male, who succumbed to *P aeruginosa* mastoiditis. Severe inflammatory changes were observed in the facial nerve and the tensor tympani muscle; the temporal bone showed necrotic changes. Very little reaction was noted in the inner ear Although pericapsulitis was striking both in the small vessels as well as in the internal carotid artery and the jugular bulb the intima appeared intact and thrombotic changes were absent.

Present series

During the course of the past year we observed 5 patients suffering from pseudomonal granulomatous external otitis. Four were males, aged 61 65 69 and 75 years and one female aged 68 years. The latter patient as well as one man presented a concomitant, moderately severe diabetes. In all the patients the clinical picture was that of external otitis refractory to routine treatment. The patients were referred to us because of persistent pain and aural discharge of 1–3 months duration. In all, granulation tissue was observed on the meatal floor at the junction of cartilaginous and osseous parts. Culture of the pus yielded a growth of *P aeruginosa*. Treatment included administration of Garamycin (gentamycin sulfate Schering Corp. Bloomfield, N.J., USA) intramuscularly and topical applications of Coliracin. In 2 patients, both diabetics, severe pain and marked local inflammatory signs prompted us to excise the granulation tissue with adjacent cartilage and leave the wound wide open, dressed with appropriated antibiotics. Four patients made an uneventful recovery One man developed the malignant" form of the disease and will be described in detail.

CASE HISTORY

A 69-year-old farmer was referred to our department on November 23rd, 1969 because of severe pain in the right ear associated with purulent discharge, of 3 months duration.

Physical examination showed the patient in a good general condition. A polypoid mass was observed in the right auditory meatus. Laboratory tests revealed blood sedimentation rate of 40/90 (Westergren) and an elevated white blood cell count (11 000/mm³) Blood sugar urea electrolytes, urinalysis, as well as EKG were within normal limits. X-ray examination of the mastoids showed moderate sclerotisation on the diseased side. Bacteriological culture of the aural discharge indicated the growth of *P aeruginosa* sensitive to Garamycin and Carbenecillin and slightly sensitive to chloramphenicol.

The patient received systemic treatment with Garamycin and chloramphenicol, as well as topical instillations of chloramphenicol. On the third day of hospitalisation, a right complete peripheral facial palsy supervened. The patient was operated upon and the polypoid mass was excised, it appeared to originate from the floor of the external meatus, at the junction of the cartilaginous and bony parts. At this site a probe could be introduced for a distance of 2 cm in the direction of the parotid gland. The ear drum appeared normal. Control of the antrum showed no pathological changes.

Signs of facial nerve palsy disappeared on the fifth postoperative day However chondri-

Acta Otolaryng 73 374–378, 1972

PSEUDOMONAL GRANULOMATOUS EXTERNAL OTITIS

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(Received June 7 1971)

Abstract Five patients all in their seventh or eighth decade suffering from external otitis due to *Pseudomonas aeruginosa* are described, four men and one woman. Concomitant diabetes was observed in two. Four patients recovered following systemic antibiotic therapy and limited local excision, and one died in spite of a similar treatment schedule. His cardiovascular system showed few signs of aging and he had no diabetes. The invasive nature of this entity is stressed. Its causative background appears enigmatic.

Pseudomonas aeruginosa is a gram-negative, rod-shaped organism, strictly aerobic, which produces a blue or green water-soluble pig It is widely distributed in nature, com-y found in water and soil, and sometimes in the human intestinal canal and on normal skin (Wilson & Miles, 1964) It is absent from the normal aural flora but may be associated with local suppuration in the external or middle ear as well as in the nose. The organism was thought to flourish mainly as a secondary invader but recently it has been described as a primary pathogenic agent.

Pseudomonas aeruginosa is usually of low virulence but it may acquire pathogenic features, especially in a tropical, humid climatic environment. The infection may be acquired in a hospital following surgery severe burns (Chandler 1968) or treatment by broad-spectrum antibiotics, corticosteroids and antimetabolites (Williams et al., 1960). Destructive lesions may appear on the skin, called ecthyma gangerosum. Infants, especially premature or newborn are prone to develop severe temic infection.

The pathological picture is characterised vasculitis of venules and arterioles, associ with haemorrhages, thrombo-embolic phenomena and tissue necrosis.

If uncontrolled and not satisfactorily drained, infection of the external auditory meatus may spread to produce necrotising chondritis and osteomyelitis of the temporal bone. The inflammatory process often traverses the intercartilaginous dehiscences of the external meatal floor and penetrates the parotid loge, the temporomandibular joint and the soft tissues of the neck. The most important diagnostic sign is persistent granulation tissue on the floor of the external auditory canal near the junction of the cartilaginous and the bony portions. This finding requires immediate evaluation including culture studies, biopsy search for diabetes, X-ray of the mastoids and the skull. If the diagnosis of pseudomonal external otitis is established early surgical debridement becomes mandatory as well as massive therapy with appropriate antibiotic agents (Chandler 1968).

Previous clinical experience

Meltzer & Kelemen (1959) were the first to describe the histological findings in osteomyelitis of the temporal bone zygoma and mandible, caused by *Pseudomonas aeruginosa*.

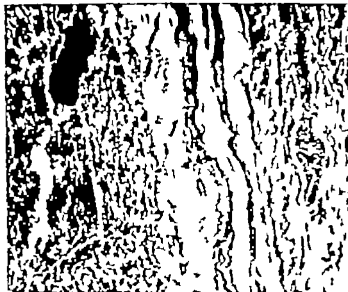


Fig. 3 Inflammatory infiltration involving the facial nerve.

Inspection of the right temporal bone revealed extensive destruction associated with purulent exudate (Fig. 1). The petrous apex was completely eroded. Purulent discharge extended from the apex to the trigeminal impression, the internal auditory meatus and the area of the Eustachian tube. The erosive, purulent process invaded the jugular fossa and produced thrombosis of the superior bulb of the internal jugular vein. The thrombosis extended into the sigmoid sinus as well as along the internal jugular vein.

Histological examination of the middle and the inner ear showed dense inflammatory infiltration composed mainly of lymphocytes and plasma cells and a small number of neutrophils. Osteomyelitic changes were seen in the great part of the petrous and mastoid bones (Fig. 2). Perineural inflammatory infiltration was observed in the vicinity of the

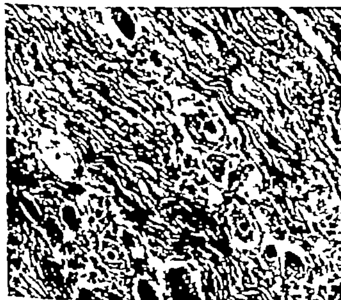


Fig. 4 The trigeminal ganglion involved by inflammatory infiltration.

vertical section of the facial nerve (Fig 3). Similar changes involved the trigeminal and the glossopharyngeal ganglia (Fig. 4) Edema of the brain was prominent.

The heart was of normal configuration and size. The coronary arteries were permeable their endothelium smooth. The aorta appeared intact except for a small, single arteriosclerotic plaque in its thoracic part.

No pathological changes were observed in the kidneys, the pancreas and the other organs.

Death was probably due to cerebral edema.

DISCUSSION

Chandler was impressed (1968) by the high mortality rate in pseudomonal external otitis and termed the disease "malignant" It may be observed that in some of Chandler's cases, death was apparently due to associated cardiopulmonary pathologic conditions. Our experience points to two rather distinct forms of pseudomonal infection of the external meatus. One may be amenable to intensive specific antibiotic therapy but persistent pain, granulations and discharge require local excision as advocated by Chandler (1968). The other malignant" form perhaps more appropriately described as "invasive" appears to be rare. Its etiological background remains obscure. In our patient the cardiovascular system was in perfect condition and he suffered from no disease other than the external otitis. The invasive nature of the inflammatory process was evident prior to any surgical treatment. Hence it is difficult to assess the danger of opening vascular routes for the spread of the disease by surgical procedure on the temporal bone. In our mind, one should avoid exposure of the temporal bone spaces, and limit surgery to the soft parts and the cartilaginous structures, unless bony involvement is evident.

ZUSAMMENFASSUNG

Es werden fünf Patienten — vier Männer und eine Frau — im Alter zwischen 70 und 80 Jahren beschrieben, die an Otitis externa — hervorgerufen durch *Pseudomonas aeruginosa* Infektion — litten. Bei zwei Kranken lag gleichzeitig ein Diabetes mellitus vor Vier Patienten wurden durch systematische Antibiotica Therapie und lokal begrenzte Excision geheilt. *Ein Patient starb trotz gleichartiger Therapie. Sein* Cardiovasculäres System zeigte wenige Altersveränderungen, Diabetes lag bei ihm nicht vor Es wird besonders auf die angreifende Natur dieser klinischen Einheit hingewiesen Ihr verursachender Hintergrund erscheint rätselhaft.

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Acta Otolaryng 73 379–386 1972

OBSERVATIONS ON THE EFFECT OF CONTRALATERAL NOISE ON INTENSIVE DIFFERENTIAL SENSITIVITY

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(Received June 7 1971)

Abstract. Intensity DLs were obtained on normal hearing subjects at 500 and 4 000 Hz using two temporally different stimulus patterns in quiet and in the presence of a contralaterally presented noise. The DLI was found to be influenced by the noise, but whether the DLI became larger or smaller appeared at first to be dependent on the temporal pattern of the measurement stimulus. A second experiment, however established that the effect of a contralateral noise on DLI magnitude may be more related to the proximity of background onset to increment onset than to the temporal nature of the background *per se*

The effects of contralateral stimulation on absolute threshold (Wegel & Lane 1924 Palva, 1954 Ingham, 1957 Sherrick & Mangabeira-Albernaz, 1961 Martin et al., 1965) on monaural loudness judgments (Prather 1961 Vigran, 1965 Rowley 1966) and on Békésy tracing excursion width (Dirks & Malmquist, 1965 Dirks & Norris, 1966 Blegvad & Terkildsen, 1966 Blegvad, 1967) have been well documented. Additionally two reports have suggested that noise presented to the opposite ear may also affect the magnitude of the monaural difference limen for intensity or DLI (Chocholle & Saulnier 1962 Blegvad & Terkildsen, 1967).

Both investigations involved the detection of amplitude increments superimposed on a background tone. In the experiment conducted by Blegvad & Terkildsen (1967) increments were mounted on a continuous background tone while a continuous thermal noise was presented to the opposite ear The result was a reduction in the size of the DLI compared to the values obtained in quiet at 1 000 and 4 000 Hz, two of the three frequencies tested. In the study undertaken by Chocholle & Saulnier (1962) the background tone and contralateral noise were terminated immediately after the detection of each increment. The DLI observed under these conditions tended to be larger than that obtained in quiet at 1 000 Hz, the only frequency tested.

Thus, while the two experimental paradigms resembled one another in certain respects, they differed significantly in at least one important particular Specifically both experiments employed what may be thought of as variants of the quantal stimulus pattern in which increments were superimposed on a background of self tone, but in one experiment the background tone was presented continuously while in the other the background tone was interrupted between increment presentations. When considered together the studies suggested the possibility that a contralaterally presented thermal noise might affect the magnitude of the DLI differently depending upon whether the background was continuous or discontinuous in character Experiment I was designed to explore this possibility

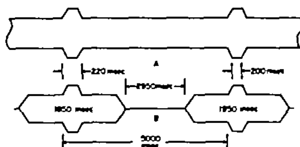


Fig 1 Diagram of the signal envelopes used in Experiment I (*A*) Continuous background with increments. (*B*) Interrupted background with increments.

EXPERIMENT I

Experimental stimuli

Two different stimulus patterns were used to measure the monaural DLI. One pattern consisted of a continuous background tone of 120 sec duration upon which 20 amplitude increments were superimposed at 5 sec intervals. The other pattern consisted of an interrupted or discontinuous, background tone made up of 20 brief tonal pedestals separated by intervals of silence. This pattern recycled for a period of 120 sec during each test run. A single amplitude increment was centered on each pedestal. The increments and backgrounds for both stimulus patterns were of the same frequency and phase. The characteristics of the signal envelopes are represented diagrammatically in Fig 1

The contralateral stimulus was thermal noise band-limited by the characteristics of the earphone (TDH 39) used to transduce it. The introduction of the noise occurred simultaneously with the initiation of the tonal background pattern in the test ear and after 120 sec the noise was terminated concurrently with the end of the tonal background.

Subjects and experimental apparatus

Eight normal young adults served as subjects. Each subject was required to pass a pure tone screening test administered at 15 dB hearing level (ANSI 1969) at octave intervals between 250 and 8 000 Hz.

A simplified block diagram of the apparatus comprising the signal and noise channels is presented in Fig 2. A more elaborate de-

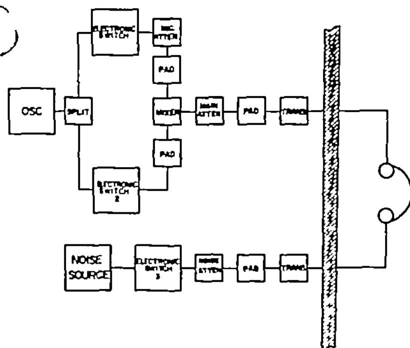


Fig 2 Simplified block diagram of the experimental equipment comprising the signal and noise channels.

Table I. *Individual averaged DLI estimates in dB for each subject under each experimental condition and mean DLIs for each experimental condition averaged over all eight subjects in Experiment I*

	500 Hz				4 000 Hz			
	Continuous		Interrupted		Continuous		Interrupted	
Subject	Quiet	Noise	Quiet	Noise	Quiet	Noise	Quiet	Noise
1	1.243	1.225	1.210	1.545	1.206	1.062	1.400	1.605
2	0.967	1.100	0.853	0.959	0.742	0.683	0.944	0.988
3	1.305	1.310	1.071	1.278	0.918	0.697	0.813	0.784
4	0.951	1.161	0.769	1.079	0.725	0.585	1.075	1.115
5	0.732	1.083	0.674	0.880	0.465	0.491	0.737	0.805
6	1.493	1.281	0.973	0.904	0.436	0.442	1.050	1.344
7	0.931	0.839	0.865	1.122	0.830	0.638	1.018	1.253
8	1.090	0.940	0.842	0.874	0.628	0.554	0.767	1.141
Grand mean	1.089	1.117	0.907	1.080	0.744	0.644	0.976	1.129

scription of this equipment along with details of the timing and programming apparatus is presented elsewhere (Paul, 1969).

Procedure

Estimates of DLI magnitude were obtained for each subject at 500 and 4 000 Hz. The background stimulus was presented at 60 dB SPL at both test frequencies. Measurements at each frequency were obtained both in quiet and in the presence of a continuous white noise presented at 60 dB SPL. The difference limen for intensity was measured in the right ear of each subject and the noise was presented to the left ear. The order of presentation of the test frequencies, the stimulus patterns and the noise were counterbalanced in order to reduce the effects of systematic bias.

In order to guard against false positive responses the programming apparatus defined a specific response interval following each increment presentation and provided for the random insertion of four catch-trials during which an increment was withheld. A response made outside the response interval or during the catch-trial presentation immediately terminated an experimental run and a new run was initiated.

Twenty increments of a single magnitude were presented during each experimental run. The responses obtained for two different increment magnitudes were used to plot psychometric functions. The two increment values used for each function were selected in-

Table II. *Summary of analysis of variance comparing the DLIs measured in quiet with those measured in noise for each combination of background and frequency*

Source	Degrees of freedom	Sum of squares	Mean square	F
Quiet vs noise within continuous at 4 000 Hz	1	0.040	0.040	10.00[a]
Subjects noise level within continuous at 4 000 Hz	7	0.028	0.004	
Quiet vs noise within interrupted at 4 000 Hz	1	0.095	0.095	9.50[a]
Subjects noise level within interrupted at 4 000 Hz	7	0.071	0.010	
Quiet vs noise within continuous at 500 Hz	1	0.004	0.004	0.222
Subjects noise level within continuous at 500 Hz	7	0.127	0.018	
Quiet vs noise within interrupted at 500 Hz	1	0.119	0.119	11.90[a]
Subjects × noise level within interrupted at 500 Hz	7	0.070	0.010	

[a] Significant at the 0.075 level of confidence.

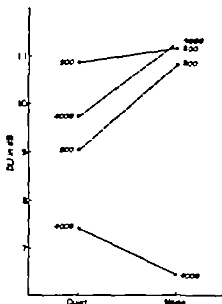


Fig 3 Plot of the mean DLI in dB for each experimental condition averaged across all subjects. Solid lines connect the DLIs obtained with the continuous background and dashed lines connect the DLIs obtained with the interrupted background. The frequency at which each DLI was obtained is indicated on each line.

dependently for each subject such that detection for one increment was less than 50% but than 20% while for the other incre- detection was greater than 50% but less 80%. Four separate functions were gen- ated by each subject under every experimental condition. The 50% points of these four functions were then interpolated and averaged to obtain the DLI estimates presented in Table I. These data were subjected to statistical treatment in a multi-factor analysis of variance procedure. A summary of this analysis is presented in Table II.

Results of Experiment I

The results of the experiment are depicted graphically in Fig. 3. The difference observed between the DLIs obtained in quiet and in noise at 500 Hz for the continuous background pattern was found to be statistically non-significant. At 4 000 Hz, however, contralateral noise caused a significant improvement in differential sensitivity ($p < 0.025$) for the continuous background pattern. In sharp contrast, the introduction of the noise under the discontinuous background condition resulted in a significant deterioration of differential sensitivity at both test frequencies ($p < 0.025$).

DISCUSSION

In general, the results appear to confirm that contralateral noise affects the magnitude of the difference limen for intensity differently depending upon whether the measurement stimulus is continuous or discontinuous in character. The nature of the mechanism or mechanisms underlying the observed behavior however is open to speculation.

Blegvad & Terkildsen (1967) suggested that contralateral noise stimulation might exert its influence on differential sensitivity by means of a process similar to adaptation. Provided that contralateral stimulation does, indeed, produce adaptive like behavior in the test ear support for their speculation might be drawn from the work of Upton & Holway (1937), Rawdon-Smith & Sturdy (1939) and Endicott (1964), all of whom have shown that adaptation significantly affects monaural intensive differential sensitivity.

Within the present experiment, adaptation might explain the large difference in differential sensitivity observed between 500 and 4 000 Hz under the continuous background condition in quiet. An increase in adaptation upon the introduction of contralateral noise might also be entertained as a possible reason for the improved DLI at 4 000 Hz under the continuous background condition with respect to the value obtained in quiet. But clearly adaptation cannot account for the significant deterioration in performance observed at both 500 and 4 000 Hz upon the introduction of noise under the interrupted background condition.

This outcome prompted a reconsideration of the temporal characteristic of the two patterns. It became apparent that aside from their simple continuous or discontinuous na-

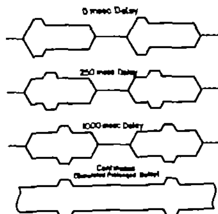


Fig. 4. Diagram of the stimulus patterns used in Experiment II. The amount of delay between background onset and increment onset is noted on each pattern.

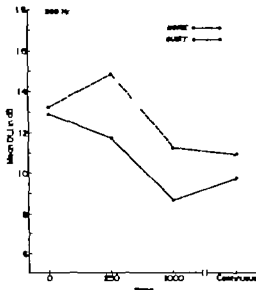


Fig. 5. Illustration of the results of Experiment II in which the mean DLIs in dB obtained at 500 Hz in quiet and with contralateral noise are plotted as a function of increment delay following onset of the background.

ture they possessed the important distinction that the temporal relationships between the onset of the background stimulus and the introduction of the increments differed radically in the two patterns.

Specifically each increment under the interrupted background condition always occurred after a brief fixed delay with respect to the onset of the background stimulus, while under the continuous background condition only the first few increments occurred close to the single onset of the background and most arose from a steady-state background. This difference suggested that the location of an increment with respect to background onset, rather than the simple continuous or discontinuous nature of the background, might be an important variable affecting the measurement of differential sensitivity.

A second experiment was undertaken to investigate this possibility directly. The specific purpose was to determine if the influence of contralateral stimulation on DLI magnitude varied as the delay between background onset and increment onset was increased.

EXPERIMENT II

Experimental stimuli subjects and procedure. The experimental stimuli resembled those used in the main experiment except for the timing of the increments. As Fig. 4 shows, these were introduced variously at zero, 250 and 1 000 msec delays with respect to the onset of the background tone. The duration of the background was maintained at 2 000 msec, the same duration as that used for the interrupted background in the main experiment. The rise time of the background was shortened from 50 to 10 msec to eliminate a discontinuity in the signal envelope during the zero-delay conditions.

To represent detection performance after a prolonged delay the continuous background was again used. While this did not represent an exact extension of the stimulus used for the other conditions, it did represent a situation in which most of the increments were temporally remote from the onset of the background, and its use made possible a substantial reduction of testing time.

Eight young adults, seven of whom had sat for the main experiment, served as experimental subjects. The stimulus patterns were

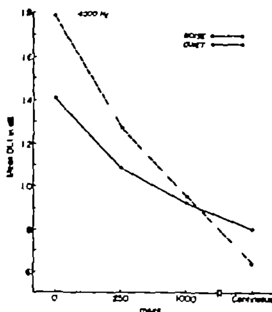


Fig. 6. Illustration of the results of Experiment II in which the mean DLIs in dB obtained at 4 000 Hz in quiet and with contralateral noise are plotted as a function of increment delay following onset of the background.

presented at 500 and 4 000 Hz, both with and without contralateral noise. The experimental apparatus was modified slightly to permit establishing thresholds for the increments by ans of the psychophysical method of adjustment

Results of Experiment II

Fig. 5 summarizes the results obtained at 500 Hz. There appears to be a tendency for the DLIs obtained both in quiet and in the presence of contralateral noise to become smaller as the onset of the increment is delayed with respect to the onset of the background tone. Under all conditions of delay the DLIs obtained with contralateral stimulation are larger than those obtained in quiet.

The results obtained at 4 000 Hz are displayed in Fig. 6. Here the curves are much smoother and show a somewhat different relationship. Clearly differential sensitivity improves progressively both in quiet and with contralateral noise, as the interval between the initiation of the background and the presentation of the increment is extended; however, rather than the rough parallelism exhibited by the curves for 500 Hz, another relationship emerges at 4 000 Hz. Under the zero delay condition the DLI obtained with contralateral noise is substantially larger than that obtained in quiet. As the interval between the introduction of the background and that of the increment is prolonged, however, the difference between the magnitude of the DLI obtained with contralateral noise and that obtained in quiet declines until, for the prolonged delay, the former becomes the smaller of the two.

DISCUSSION

Although a different psychophysical procedure was used in Experiment II the direction and magnitude of differences between the DLIs obtained in quiet and noise under the 1 000 msec delay and continuous background conditions remains approximately the same as those obtained in Experiment I. It would appear then that the changes in technique introduced in Experiment II did not alter the effect of contralateral noise on DLI size.

The results of Experiment II appear to show that contralateral noise exerts its influence on increment detection by producing an initial disruptive effect which eventually subsides as the onset of the increment is delayed with respect to the initiation of the background stimulus. For the longest delay there appears to be a frequency-dependent effect. The DLI obtained at 500 Hz with contralateral noise tends simply to approach the value of that obtained in quiet, but at 4 000 Hz the noise appears to exert a facilitative effect on detectability resulting in a diminution of the size of the DLI compared to that obtained in quiet.

Similar, and perhaps related, effects have been demonstrated in other experiments concerning the role of temporal relationships in both ipsilateral and contralateral masking. Zwicker (1965 *a*, *b*) and Lankford & Stokinger

(1970) found more threshold shift for tone pips occurring close after the onset of a masker than when they were delayed with respect to masker onset. They referred to this effect as "masking overshoot." Zwislocki et al. (1967) and Stokinger & Lankford (1970) found that a greater threshold elevation occurred when the onset of the test signal closely followed the onset of a contralateral masker than when the two were widely separated in time.

In aggregate, these results, in addition to our own, seem to reflect the existence of a mechanism which produces an initial disruptive effect that diminishes with the passage of time. Hind et al. (1963) have described somewhat parallel behavior in certain of their investigations of neural spike activity in the inferior colliculus of the cat during monaural and binaural acoustic stimulation.

Some of their observations suggest that a stimulus which causes a net inhibitory result may exert, at least for an initial period of time an effect which is usually interpreted as the result of excitation. To quote these authors, "Thus, when binaural stimulation results in fewer spikes than are produced by monaural stimulation, the binaural stimulation may nevertheless cause an onset burst with a shorter latent period and probably a larger number of spikes in the onset burst than is the case for the monaural excitatory stimulus."

If increment detectability varies with background neural activity level as Endicott (1964) suggests, it is possible that contralateral stimulation exerts its influence on detectability by altering the level of such activity through the mechanism of binaural excitatory-inhibitory interplay described by Hind and his coworkers.

If in the present experiments, contralateral noise did indeed, cause a more vigorous neural response to the onset of a background stimulus than that produced by the background alone, the increased amount of neural activity resulting from this interaction might have rendered a closely following increment more difficult to detect during the interrupted background condition. During the continuous background condition, on the other hand, the initial burst of neural activity soon would have subsided into a net inhibitory effect so that while the detectability of the first few increments in a series might have been affected adversely by the onset activity that of the remaining increments might have been enhanced by virtue of the reduction of neural activity attributable to inhibition coupled with that attributable to adaptation. It is apparent that the results obtained at 4 000 Hz in Experiment II fit this model better than do those obtained at 500 Hz.

CONCLUSIONS

The notion that the results of the experiments may largely be explained on the basis of a binaurally-produced excitatory-inhibitory interplay of neural elements coupled with adaptation is, of course, entirely speculative. The results, themselves, however amply demonstrate that the introduction of contralateral noise significantly affects increment detectability They indirectly support the findings of Blegvad & Terkildsen (1966) that the SISI (Short Increment Sensitivity Index) score may be significantly affected by a contralateral masker For example, when our data are transposed into an expression representing per cent detectability for a given increment size, the introduction of contralateral noise is seen to result in improvements in detectability from subject to subject ranging from 6–32% at 4 000 Hz under the continuous background condition.

Since, in the clinical assessment of auditory function, it is often necessary to deliver a masking noise to the non-test ear to prevent its participation in the measurement task, it is ironic that a sizeable body of experimental evidence is accumulating which demonstrates that stimulation of the opposite ear in this manner significantly affects the performance

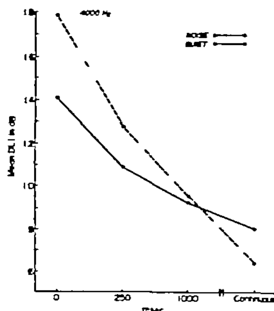


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The results obtained at 4 000 Hz are displayed in Fig. 6. Here the curves are much smoother and show a somewhat different relationship. Clearly differential sensitivity improves progressively both in quiet and with contralateral noise, as the interval between the initiation of the background and the presentation of the increment is extended; however rather than the rough parallelism exhibited by the curves for 500 Hz, another relationship emerges at 4 000 Hz. Under the zero delay condition the DLI obtained with contralateral noise is substantially larger than that obtained in quiet. As the interval between the introduction of the background and that of the increment is prolonged, however the difference between the magnitude of the DLI obtained with contralateral noise and that obtained in quiet declines until, for the prolonged delay the former becomes the smaller of the two.

DISCUSSION

Although a different psychophysical procedure was used in Experiment II the direction and magnitude of differences between the DLIs obtained in quiet and noise under the 1 000 msec delay and continuous background conditions remains approximately the same as those obtained in Experiment I. It would appear then that the changes in technique introduced in Experiment II did not alter the effect of contralateral noise on DLI size.

The results of Experiment II appear to show that contralateral noise exerts its influence on increment detection by producing an initial disruptive effect which eventually subsides as the onset of the increment is delayed with respect to the initiation of the background stimulus. For the longest delay there appears to be a frequency-dependent effect. The DLI obtained at 500 Hz with contralateral noise tends simply to approach the value of that obtained in quiet, but at 4 000 Hz the noise appears to exert a facilitative effect on detectability resulting in a diminution of the size of the DLI compared to that obtained in quiet.

Similar and perhaps related, effects have been demonstrated in other experiments concerning the role of temporal relationships in both ipsilateral and contralateral masking. Zwicker (1965 *a*, *b*) and Lankford & Stokinger

Acta Otolaryng 73 387–390 1972

COMBINED EFFECT OF NOISE AND NEOMYCIN ON THE COCHLEA

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(Received June 7 1971)

Abstract. The study was designed to examine the combined harmful effect of intensive noise and neomycin on the cochlea in three groups of guinea pigs; one receiving neomycin alone, one exposed to noise alone, and one exposed to both factors. Electrophysiologically and microscopically the harmful effects were shown, when the two factors were combined, to increase more than predicted by simple addition of the two effects. The correlation between the per centage of destroyed outer hair cells and the microphonic potential amplitude loss was calculated.

Ample experimental and clinical evidence has accumulated concerning the hazardous effects of noise and of ototoxic antibiotics on the cochlea. Both produce morphologically obser vable changes such as hair cell damage and reduction of amplitudes in bioelectric potentials of the cochlea (Eldredge & Cowell, 1958 Beagley 1965 Kohonen, 1965 Stockwell et al., 1969) Studied by light microscopy the damage caused by noise and ototoxic antibiotics is characterized by degeneration of the sensory cells of the organ of Corti. The outer hair cells are known to be more vulnerable than the inner hair cells, whatever the noxious agent may be The extent of the lesion on the cochlear partition is determined by the charac teristics of the noise and the duration of ex posure, or in cases treated with antibiotics, by the dosage of the noxious drug.

The noisy environments in modern society have stimulated interest in studying the combined effect of these two factors. Even in clinical practice situations may arise where a patient is exposed to both noise and a chemical ototoxic agent. Experimental studies of the combined effect of these two noxious agents, applied simultaneously or in succession, are reported below

MATERIAL AND METHODS

The study to be reported consists of four series of experiments on guinea pigs. The first group includes 10 animals exposed only to noise of some 115 dB overall sound pressure level (re 2×10^{-5} N/m²) for an average of 70 hours. The second group, 8 animals, were given neomycin 200 mg/kg i.m. during 7 days. The third group, 8 animals, were exposed both to noise and to neomycin using the same dosages as in the above two groups. After receiving the neomycin they were exposed to noise for the 70 hours. The fourth group, six ears, served as controls.

Noise exposure was produced in free field conditions with a loudspeaker in close proximity The animals were allowed to move freely in their cage. They were given food and water during exposure. The noise consisted of filtered white noise with a central frequency of 8 000 Hz and one octave bandwidth. The sound pressure level was checked each time by a precision sound level meter (Brüel & Kjaer model 2203) in linear position. In the first group of exposures the sound pressure level was checked by continuous graphic recording using the Brüel & Kjaer

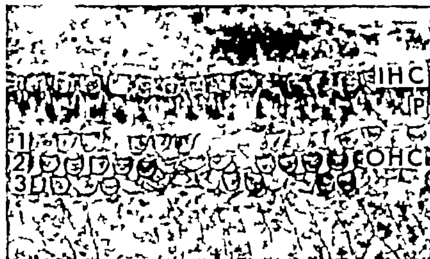


Fig 1 Surface specimen from basal coil of neomycin-treated animal. Degenerated outer hair cells stand out distinctly among the normal ones. *IHC* = inner hair cells; *1, 2, 3* = three rows of OHC = outer hair cells; *P* = inner and outer pillar cells.

frequency analyser and recorder (Type 3314). A high frequency bandwidth was chosen in order to obtain a noise damage in the basal turn of the cochlea, the same region where hair cell damage is maximal after application of neomycin.

About 2 weeks after cessation of noise or neomycin application, the animals were anaesthetized intraperitoneally with pentobarbital sodium (30 mg/kg). The ear was prepared or round window recording of cochlear potentials. The potentials were ...ed up by silver electrodes, amplified by a preamplifier (Tektronix 122) and potential amplitudes were measured by the Brüel & Kjaer frequency analyser (Type 2017) in combination with an octave filter set (Type 1612). The potential amplitude values were read at the test frequencies 250, 500, 1 000, 2 000 and 4 000 Hz and the sound pressure levels 60, 70, 80, 90 and 100 dB. The sound stimuli were fed to the ears of the guinea pigs from a hearing aid earphone through a plastic tube sutured to the pinna. The sound pressure levels were calibrated using an artificial ear model 9 A, with a 2 cm³ coupler

After electrophysiological recordings the temporal bones of the animals were removed

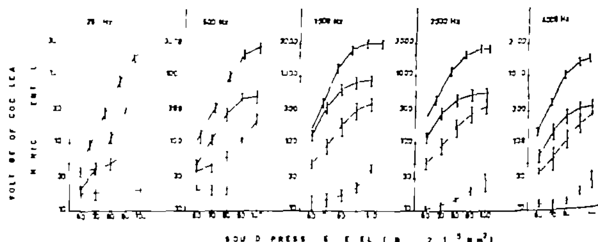


Fig 2. Cochlear microphonic potentials obtained from four groups of animals. The top curve refers to the normal control group, the second—showing somewhat decreased values—to animals exposed to neomycin alone, the third to animals exposed to noise alone, and the bottom curve to animals exposed to neomycin and noise.

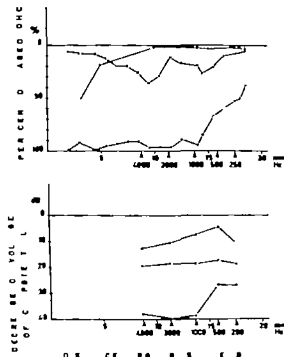


Fig 3 Graphic presentation of the histological sensory cell loss (upper diagram), and decrease of cochlear microphonics (lower diagram) in animals exposed to neomycin (●), to noise alone ( ), and to neomycin and noise (o). In the lower diagram the losses in cochlear microphonic potentials were obtained at 80 dB SPL.

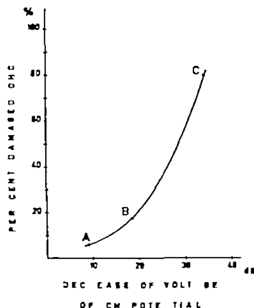


Fig 4 Relation between percentage outer hair cell damage and average loss in cochlear microphonic potentials. Point *A* refers to animals exposed to neomycin, point *B* to those exposed to noise alone, and point *C* to those exposed to both noise and neomycin.

for histological study The microdissection technique developed by Engström et al. (1966) was used. In the case of the animals exposed to noise and those exposed to both noise and neomycin, the whole organ of Corti was dissected free, if technically possible, and mounted on glass slides for study as "sur face specimens" (see Fig. 1). In the case of neomycin-treated animals a sample of approx mately 1/3 turn was dissected from each coll of the cochlea. From the specimens, areas of the organ of Corti corresponding in length to 50 outer hair cells in one row were syste matically mapped and the sensory cell loss re gistered in so-called cochleograms (Kohonen, 1965 Engström et al., 1966). The position of the mapped areas on the basilar membrane is shown in Fig. 3, upper diagram. Thus eight such areas per organ of Corti were examined from the ears of the neomycin-treated animals, and 18 areas per ear from the animals exposed to noise alone and to neomycin plus noise (Fig. 3). The numerical distances from the stapes on the cochlear partition are quoted according to Ward & Fernández (1961).

RESULTS AND DISCUSSION

The mean amplitudes of the cochlear microphonic potentials (see Fig. 2) for the different test frequencies show a loss for the two groups of noise exposure and neomycin exposure alone. When plotting the potential amplitudes in μV on a logarithmic scale, the losses obtained in the third group with combined noise and neomycin exposure appear greater than the two losses combined. Compared with the normal control group all the losses are of statistical significance.

The hair cell lesions are illustrated in the upper diagram of Fig. 3 which shows the per centage of destroyed outer hair cells

Act

ous sites of the cochlear partition. Neomycin produces maximal damage at the basal end. The effect of noise exposure extends further up towards the apex. The combined effect shows far greater damage than might be ex pected on the basis of simple addition of the two effects.

In the lower diagram of Fig. 3 the losses of potential amplitudes are also plotted in dB (re control group) at sites corresponding to the amplitude maxima on the cochlear partition as calculated by Greenwood's function (Greenwood, 1961).

When all three groups are studied together a correlation is obtained between the proportion of outer hair cell damage and the decrease of amplitudes of cochlear microphonic potentials in dB The curve expressing this correlation (Fig. 4) indicates that a relatively great loss in potential amplitudes results from comparatively slight hair cell damage. This correlation does not fit exactly into a linear relation when plotted on a log-log-scale.

The combined effect of noise and neomycin on the cochlea, as studied by electrophysiological and histological methods, shows that of the two factors increases the effects the other This suggests that sensory cells ized by a chemical noxious agent such as neomycin, are more susceptible to mechanical trauma, for example intensive noise. Although these drugs are usually given to hospitalized patients only there seems to be some reason to assume greater susceptibility to noise-induced hearing loss in patients treated with these antibiotics.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial help given by the Finnish National Council for Medical Sciences. We also wish to thank Mr Eero Lampio, Dr techn., and Mr Jarmo Toivanen, M.Sc., from the Laboratory of Acoustics of the Helsinki University of Technology for their help and for granting us the facilities for noise exposure and measurement.

ZUSAMMENFASSUNG

Die Aufgabe dieser Arbeit besteht aus einer Untersuchung über den kombinierten Einfluss von Lärm und Neomyzin auf die Schnecke. Dafür wurden drei Gruppen von Meerschweinchen benutzt. Der ersten wurde Neomyzin gegeben, die zweite wurde Lärm ausgesetzt, und die dritte wurde mit Neomyzin und Lärm behandelt. Kombinierte elektrophysiologisch und mikroskopisch nachweisbare Änderungen traten häufiger auf, als vorher auf Grund einfacher Addition vermutet worden war Das Verhältnis zwischen gestörten Haarzellen in Prozent zu dem Abfall der Mikrophonpotentialamplituden wurde berechnet.

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Acta Otolaryng 73 391–393, 1972

FREQUENZANALYTISCHE, TIEREXPERIMENTELLE STUDIE DER CM NACH HISTAMINGABE BEIM MEERSCWEINCHEN[1]

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(Eingegangen am 25 November 1971)

Abstrakt. An 15 Tieren wurden frequenzanalytische Untersuchungen der CM vor der Injektion von Histamin durchgeführt. Die Frequenzanalysen wurden 2, 10 und 20 Minuten nach der Injektion von Histamin wiederholt. Die nach der Injektion erhaltenen Meßwerte wurden mit denen vor der Injektion verglichen. Die Untersuchungen ergaben Diagramme, wie sie bei der Menière'schen Krankheit gefunden werden.

Die Genese der Menière schen Krankheit (M.K.) und die medikamentöse Therapie stützen sich häufig auf das biogene Amin, das Histamin. Das Vorkommen dieser Erkrankung bei Patienten mit allergischen Leiden wurde wiederholt mitgeteilt (Atkinson, 1941 1944 Kallos & Kallos-Deffner 1952, Kämmerer 1954)

Bei diesen Patienten kommt es durch Antigen Antikörperreaktionen zur vermehrten Freisetzung von Histamin. Es gelingt in vielen Fällen bei derartigen Erkrankungen die Menière Anfälle durch Antihistaminica zu kupieren.

Bei der medikamentösen Therapie der M.K. werden durch die Anwendung von Histamin nahezu ausnahmslos (Ismail, 1959) positive Ergebnisse berichtet (Sheldon & Horton, 1940 Giaccai & Gardengki, 1951 de Vido, 1954 Krejci, 1955 Meyer zum Gottesberge 1970)

Es wurde deshalb in einer ersten tierexperimentellen Studie das Verhalten der CM nach der Injektion von Histamin am Meerschweinchen untersucht und ein unterschiedliches Verhalten beobachtet. Bei der Registrierung der CM mit der Meßfrequenz 2 kHz, Schallpegel 60 dB (über 2×10^{-4} µbar) wurde bei 23 Tieren 13 mal ein Abfall, 5 mal ein Gleichbleiben und 4 mal ein Anstieg beobachtet (Nowak & Dahl, 1967)

Da durch diese erste Versuchsserie keine Aussage zum Verhalten der einzelnen Frequenzen möglich war sollten die CM nach der Gabe von Histamin frequenzanalytisch untersucht werden.

MATERIAL UND METHODE

Versuchstier war das Meerschweinchen. Die Versuchstiere eines Inzuchtstammes waren ohrgesund und wogen 400 g. Die Präparation und die Ableitung der CM erfolgte in der üblichen Weise (Nowak & Dahl, 1967) Vor jeder Histamininjektion wurde der Frequenzgang der CM aufgezeichnet. Die nach 2, 10 und 20 Minuten post injectionem mit 2,5 mg Histaminchlorhydrat[2]/kg Körpergewicht s.c. gefundenen Frequenzgänge zeigten erhebliche Abweichungen von den Ausgangswerten.

ERGEBNISSE

Die Tiere wurden nach dem CM-Verlauf in Gruppen zusammengefaßt.

[1] Herrn Prof. Dr K. Dietzel zum 60 Geburtstag in Verehrung gewidmet.

[2] Histaminchlorhydrat[2] VEB Ankerwerk, Rudolstadt, DDR.

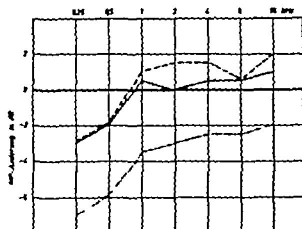


Abb 1 Versuchstier Nr 9 (Gruppe IV). Relativer Frequenzgang der Mikrophonpotentiale (MP). — 2 min. post injectionem (p.i.). 10 min. p.i. - - 20 min. p.i.

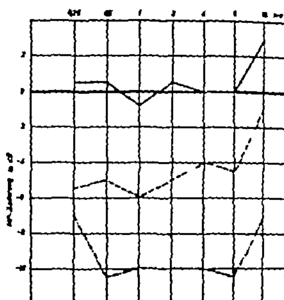


Abb 3 Versuchstier Nr 12 (Gruppe III). Relativer Frequenzgang der CM. — 2 min. post injectionem (p.i.). 10-12 min. p.i. -·- 20 min. p.i.

I Gruppe — Bei 1 Tier zeigt sich keine Beeinflussung der CM nach Histamingabe

II Gruppe — Ein Anstieg über den gesamten Frequenzbereich liegt nur bei einem Tier vor

III Gruppe — Bei 4 Tieren ist ein Abfall der CM in allen Frequenzen zu finden.

Gruppe — Bei 9 Tieren wird ein Abfall und ein Anstieg einzelner Frequenzen beobachtet. Auffällig ist der bevorzugte Abfall der tiefen und mittleren gegenüber den hohen Frequenzen.

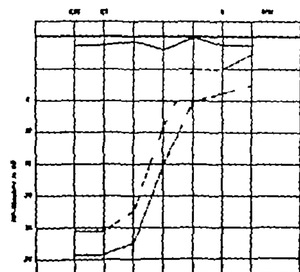


Abb 2 Versuchstier Nr 10 (Gruppe III) Relativer Frequenzgang der CM. — 2 min. post injectionem (p.i.). 10-12 min. p.i. -·- 20 min p.i.

Bei 10 Kontrolltieren ergab die Messung der CM (Frequenzanalyse) nach 2, 10 und 20 Minuten keine Abweichung vom Ausgangswert. Zur Veranschaulichung der Befunde ist der Frequenzgang einiger Tiere in einem Diagramm wiedergegeben (Abb 1-3)

DISKUSSION

In Übereinstimmung mit unseren ersten Untersuchungen über die Beeinflussung der Haarzellfunktion durch Histamin fanden wir in der Mehrzahl der Fälle einen Abfall der CM für die Frequenzen 2 kHz (12 Tiere), während 2 Tiere keine Beeinflussung zeigten. Der Angriffspunkt des Histamins liegt peripher und an den kontraktilen Elementen der glatten Muskulatur

Die Angaben über glatte Muskelfasern im M tensor tympani bei der Katze sind widersprüchlich. Während Erulkar et al. (1964) die Befunde über das Vorkommen von glatten Muskelfasern von Byrne (1938) im M. tensor tympani bestätigen, konnten Fernand & Hess

(1969) diese nicht wiederholen. Bei Ratten (Candiollo, 1967 Candiollo & Cantino 1969) und Meerschweinchen (Gerhardt et al., 1966) konnten glatte Muskelfasern im M. tensor tympani bisher nicht nachgewiesen werden, so daß wir eine Beeinflussung der vorliegenden Untersuchungsergebnisse in diesem Zusammenhang zunächst ablehnen möchten.

Eine Gefäßerweiterung in der Peripherie führt zu einer Veränderung der Kreislaufsituation. Durch eine Steigerung der Permeabilität der Gefäße kann das Histamin direkt im Gewebe selbst wirksam werden. Daneben ist aber auch ein Histaminspasmus der Gefäße bekannt

Der Abfall der CM in den tiefen und mittleren Frequenzen wäre durch eine Veränderung der Durchblutungsgüte der Gefäße in diesem Frequenzbereich des Innenohres zu erklären. Kommt es zu einem Absinken der CM des gesamten Frequenzbandes, dann könnte das Histamin nach Übertritt aus der Stria vascularis in die Endolymphe direkt an dem Neuro-Epithel des Cortischen Organs wirksam werden. Die gezeichneten Diagramme des Verlaufs der CM nach Histamingabe sprechen für eine Beteiligung des Histamins an der M.K. Da aber klinisch über eine Besserung der M.K. nach der Behandlung mit Histamin berichtet wurde, dürfte es sich bei diesem Krankheitsbild um ein wesentlich komplexeres Geschehen handeln als bisher angenommen wird.

SUMMARY

In 15 guinea pigs, the frequency of cochlear microphonics was investigated before injection of histamine and compared with amplitudes of cochlear potentials 2, 10 and 20 minutes after histamine treatment. These observations showed that histamine gave rise to frequency curves typical of Menière's disease.

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Acta Otolaryng 73 394–401 1972

ULTRASTRUCTURE OF THE MIDDLE-EAR MUCOSA IN SECRETORY OTITIS MEDIA

I Serous Effusion

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(Received June 10 1971)

Abstract. Mucosal biopsies from the middle ear of patients with secretory otitis media and serous effusion were studied by electron microscopy This confirmed that the effusion is produced by active secretion from an epithelium without signs of degeneration: Two types of secretory cell were demonstrated, one with mucigen-like granules and one with dark secretory granules and apocrine secretion, possibly serous-secreting. Compared with the normal middle ear mucosa, the number of secretory cells had increased while the number of ciliated cells had decreased. There were signs of ciliated cells being transformed into secretory cells.

Recent light and electron microscopic studies have revealed that the mucosa of the middle ear is a modification of the upper respiratory mucosa with active secretory elements (Sadé, 1966 Hentzer 1969 1970· Hussl & Lim, 1969 Kawabata & Paparella, 1969 Lim, 1970) This has thrown new light on the pathogenesis of middle-ear diseases, primarily secretory and chronic suppurative otitis media. Numerous authors have dealt with the problems concerning the origin of middle-ear effusion in secretory otitis. The majority have concluded that apparently it is formed by purely passive transudation of fluid from vessels in the middle ear while a few believe that active secretion occurs from the epithelium The first ultrastructural observations of the epithelium in experimentally induced secretory otitis media in animals are now available The first study (Lim & Hussl 1970) confirmed the secretory origin, whereas the second one (Paparella et al., 1970) showed that passive transudation seemed to be the main factor

The first ultrastructural study of the human middle-ear mucosa in secretory otitis media will be reported below

METHOD AND MATERIAL

The material comprised mucosal biopsies from 5 children, aged 3–16 years, with symptoms and signs of secretory otitis, i.e. conductive hearing loss and a flat curve in impedance measurement. The symptoms and signs had been present for periods ranging from 3 months to 3 years, average about 1 year Two patients had previously been treated by paracentesis and tubulation. At operation all 5 patients had a clear serous fluid in the middle ear After paracentesis and removal of the secretion by suction a biopsy was taken with small forceps from the promontorial mucosa. Bleeding was slight and did not give rise to any symptoms later The procedure was completed by the insertion of a tube into the tympanic membrane. The specimen was immediately fixed in cold 6.5% glutaraldehyde for 24 hours and post-fixed in cold buffered 2% osmium tetroxide for 2 hours. After stepwise



Fig. 1. Middle ear mucosa in secretory otitis media. The epithelium is pseudostratified columnar with many cilia and goblet cells (*arrows*). Anoptral Contrast ×1 900.

dehydration the specimens were passed through propylene oxide and embedded in Epon 812. Thick sections were stained with toluidine blue or were observed unstained using Anoptral Contrast. Thin sections were stained with uranyl acetate and lead citrate and studied under the electron microscope using magnifications of from ×2 000 to ×30 000.

RESULTS

In the light microscope the majority of the preparations were found to be made up of a pseudostratified columnar ciliated epithelium, about half the preparations contained goblet cells (Fig. 1). In a few preparations the epithelium was lower cuboidal, with or without cilia. The epithelium did not differ essentially



Fig 2 Surface cells from the mucosa containing abundant granular reticulum and secretory granules (*arrows*) of the same appearance as found in goblet cells. *L*, lumen.

from epithelium found in previous studies on the promontory of the normal middle ear (Hentzer 1970). In one case (with a history of several years) however the epithelium had undergone metaplasia to stratified squamous epithelium In 2 of the 5 patients glands were found in the submucous layer

Electron microscopy showed the same types of cell that have been described previously in the normal human middle-ear mucosa, viz. the non-ciliated cell with or without dark granules, the ciliated cell, the goblet cell, the intermediate cell, and the basal cell (Hentzer 1970). Ciliated cells were present in smaller numbers than in the normal mucosa, and the same applied to the non-ciliated cells with dark granules. On the other hand, there was an increased number of cells with a cytoplasm of the same appearance as in the goblet cells, i.e very dark, consisting almost exclusively of granular reticulum with distended cisternae. Scattered in the cytoplasm of these cells, there were light granules with or without a central dark core in varying numbers and of varying size. The nuclei of all these cells were dark and pyknotic. These cells were rarely of the shape of goblet cells, but of the same shape as the other epithelial cells, often pushed forward



Fig. 3 (A) Ciliated and non-ciliated cells presenting apocrine secretion in the form of pseudopodia-like bulgings of the cytoplasm from the surface. The bulgings are pressed up between the cilia, which give the erroneous impression of being situated within the membrane of the bulge.

to the surface, by way of being expelled into the lumen (Fig. 2). In some preparations there was a type of cell not seen in the normal mucosa. Issuing from the surface of ciliated as well as of non-ciliated cells there were large bulgings of the cytoplasm, suggesting pseudopodia, in several cases separated from the cell by constriction and lying in the lumen (Fig. 3 A). These structures were delimited by a more or less intact cell membrane within which there was a mesh-like structure with diverse organelles (granular reticulum, ribosomes, and vacuoles) (Fig. 3 B) In the ciliated cells these bulgings were pressed up between



Fig 3 (B) Higher magnification of surface bulging possessing diverse organelles of the cytoplasm like ribosomes and vacuoles.

the cilia which thereby gave the erroneous impression of being situated within the mem brane of the bulge.

The epithelium in the submucous glands contained the same types of cell as the surface epithelium apart from the ciliated cells. Here the non-ciliated cell with dark granules was found more commonly than in the surface epithelium (Fig. 4)



Fig. 4. Epithelial cells from submucous gland. Abundant granular reticulum is seen. Arrows point to dark secretory granules (serous secreting?).

DISCUSSION

In our previous studies of the normal human middle-ear mucosa (Hentzer 1970) 5 types of cell were found. The ciliated cell, the non-ciliated cell with and without secretory granules (including the goblet cell), the intermediate cell, and the basal cell. Similar findings have been made by others (Lim & Hussl, 1969 Kawabata & Paparella, 1969) On comparison with the cellular structure of the normal middle-ear mucosa, the following differences were found in secretory otitis with serous effusion. (1) The non-ciliated cell with dark granules (serous type?) has decreased in frequency and is found most commonly in the submucous glands. (2) The non-ciliated cell with light granules with or without a dark central core (mucus-secreting?) has increased in frequency These cells rarely assume the shape of goblet

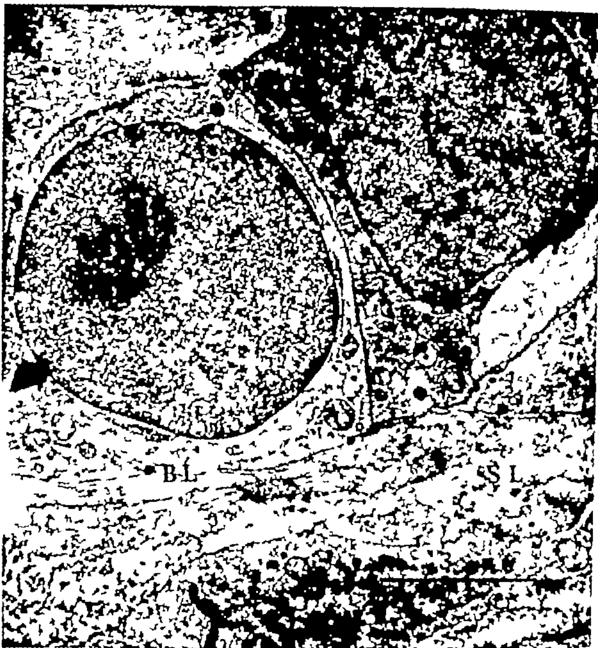


Fig 5 Two basal cells of the epithelium. The inter cellular spaces are normal, and the basal lamina (*BL*) without ruptures. *SL* submucous layer

cells, but more often that of other luminal epithelial cells and are often by way of being expelled into the lumen (3) The number of ciliated cells has decreased. (4) Lastly apocrine secretion is present, in the form of bulges sug gesting pseudopodia from ciliated as well as non-ciliated cells. While in a previous study (Hentzer 1970) we had the impression that the ciliated cell did not produce secretion in the normal middle-ear mucosa, this did occur in the mucosa in cases of secretory otitis.

Lim & Hussl (1970) has described the ultra-structure of the tympanic mucosa after tubal obstruction in the guinea pig. He found an in-creased number of the dark granulated cell and described the apocrine secretion. Papa-

rella et al. (1970) has described the ultrastructure of the epithelium in the squirrel monkey after tubal obstruction. We did not find his distended intercellular spaces, filled with a cloudy substance, which he assumed was a protein-containing fluid, and also not the rupture of the basal lamina (Fig. 5). In Paparella's opinion, this finding as well as the increased capillary permeability and the chemical similarities of the middle-ear effusion and blood serum support the theory on the hydrostatic pressure effect of passive transfer of serous fluid from the vessels to the middle ear. This theory could not be confirmed in the present material where the epithelium showed every sign of pronounced secretory activity. The cytoplasmic content of organelles was as in the normal mucosa, and the basal lamina was intact. There was also no infiltration by inflammatory cells, eosinophilic cells, or any virus-like structures in the epithelium.

It remains to be established whether the dark granulated cell and the cells having bulges suggesting pseudopodia (also described after stimulation by pilocarpine in the intestinal epithelium of man (Trier 1968) and in the parotid gland of the mouse and rat (Parks, 1962)) are responsible for the serous secretion and whether the cells with light granules produce mucous secretion. As will be shown in a subsequent publication, however, there are no major qualitative morphological differences between epithelial cells from the middle-ear mucosa of patients with secretory otitis media showing serous and mucous secretion respectively in the middle ear.

ZUSAMMENFASSUNG

Die Mittelohrschleimhaut bei serösem Mittelohrkatarrh mit serösem Exudat wurde mit dem Elektronenmikroskop untersucht. Dass das Exudat von einem aktiven Epithel ohne Degeneration produziert wurde, war bekräftigt. Zwei sekretorische Zellentypen wurden gefunden: einer mit mucigen-gleichender Granula und einer mit dunklen granulierten Zellen und apocriner Sekretion, die wahrscheinlich serös ist. Im Vergleich zu normaler Mittelohrschleimhaut wurde eine grössere Menge von sekretorischen Zellen gefunden während die ziliären Zellen weniger waren und wahrscheinlich zu sekretorischen Zellen umgewandelt werden könnten.

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Acta Otolaryng 73 402–412, 1972

PATHOLOGIC CHANGES IN IDIOPATHIC LABYRINTHINE HYDROPS

Correlations with Previous Findings

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(Received August 10, 1971)

Abstract. A pair of temporal bone specimens is presented which demonstrates the presence of labyrinthine hydrops with the components of herniated sac-like dilatations of the utricular wall, dislocation of the ampullary walls, herniation of the utricle, saccular dilatation and collapse, reparative lesions (rupture) of the saccule and utricle, hydrops of the scala media, degeneration of the organ of Corti, and ganglion cell loss. The functional and etiological significance of these pathological findings and previous conflicting reports are discussed.

Many reports have described the characteristic pathologic changes present in idiopathic labyrinthine hydrops. Since the histologic observations in a patient having had Menière's disease (Hallpike & Cairns, 1938) conflicting interpretations have been given to changes related to dilation of endolymphatic spaces, and various explanations for disturbed function have been proposed (Altmann & Kornfeld, 1965 Hallpike & Cairns, 1938 Lawrence & McCabe 1959 Lindsay 1942 Schuknecht et al., 1962) Likewise there have been conflicting reports concerning changes of the neural and epithelial elements (Hallpike & Cairns, 1938 Lawrence & McCabe 1959 Lindsay 1944 Lindsay & Schulthess, 1958 Lindsay et al., 1967 Schuknecht et al. 1962)

This work has been supported by N I H Grants NB-07007 NB-05385 NB-1330 Research Fund of the Amer Otol. Soc., Inc., and the Deafness Research Foundation.

In order to help clarify some of the previous conflicting reports, similar pathological temporal bone findings of idiopathic hydrops from a patient with probable amyotrophic lateral sclerosis are presented. Labyrinthine hydrops, sensorineural cochlear degeneration and extreme herniations of the membranous labyrinth with healed tears of the saccule and ampullary wall distortion were present in both ears. A process similar to that in the saccules was seen in the left utricle. The possibility is proposed of two separate loci of neural element changes effected by disease processes which cause sensorineural hearing loss. A graphic method allowing this interpretation is presented.

Case presentation

This 69-year-old white male was admitted to the University of Chicago Clinics because of increasing difficulty with breathing over the preceding 7 months. This difficulty was associated with coughing (non-productive), a five pound weight loss, decrease in appetite and twitching of muscles of the trunk shoulders, and upper legs. The patient had a history of progressive hearing loss for 25 years for which he wore a hearing aid. (There was no statement recorded regarding the presence or absence of tinnitus or vertigo. Later attempts at

obtaining this information from the family were unsuccessful.)

Physical examination revealed minimal diaphragm excursion (accessory muscle breathing), distant breath sounds, multiple premature ventricular contractions, and numerous fasciculations over the shoulder girdle, anterior and posterior trunk, abdomen and lower extremities.

Pulmonary function studies revealed hypoventilation and respiratory acidosis, compatible with a brain stem lesion or peripheral muscle weakness. On neurological consultation the fasciculations were noted to be associated with muscular atrophy of the intercostals, hands, arms, and legs. On consultation with otolaryngology tracheobronchitis was diagnosed at bronchoscopy. Audiometric testing showed a bilateral sensorineural hearing loss of 40 dB in the low tones to 70 dB in the high tones. Kobrak minimal caloric test responses with 80 F water were inconclusive. With 68 F water the responses were definite, the left less vigorous than the right.

The patient requested discharge from the hospital against the wishes of his physicians. At the time of his leaving the hospital he was having increased difficulty with breathing. At his home, increasing dyspnea and cyanosis developed through the night. He expired in the mid-morning hours. Autopsy was performed that day. The temporal bones were removed and placed in 20% degassed formalin and submitted to the ENT laboratories for processing.

Microscopic examination of the autopsy material showed hyalinization of striated muscle fibers with granularity, loss of striations, increase in sarcolemmal nuclei and lymphocytic infiltration. Examination of the tissue from the central nervous system revealed patchy demyelinization in the lateral horns, loss of anterior horn cells (thoracic and cervical), increase in mature lymphocytes in the anterior grey matter and degenerating Betz cells in the motor cortex. Pathologic diagnosis: undiagnosed disease of central nervous system, probably amyotrophic lateral sclerosis.

Histologic examination of the temporal bones. celloidin-imbedded, horizontal serial sections, 20 μ thick, hematoxylin-eosin stain.

Left Temporal Bone

External auditory canal and drum membrane no abnormalities were noted except for minor post-mortem autolysis.

Middle ear The mucosa was thin. There was no evidence of abnormalities.

Mastoid Extensive pneumatization. No abnormalities noted.

Cochlea. The bony labyrinthine capsule appeared normal. The cochlear duct was extremely dilated. *Reissner's membrane* pushed against the wall of the scala vestibuli, and, at the helicotrema, herniated into the scala tympani (Fig. 1). The distended *ductus reuniens* abutted the distended saccular wall in the area of the stapes footplate. Atrophy of the *stria vascularis* varied from a normal appearance or mild atrophy in the basal turns to complete atrophy in the apical area (Fig. 1). An epithelial-covered amorphous mound was seen at the apical extent of the stria. (This was not interpreted as evidence of rupture of Reissner's membrane because of its location overlying localized, partially obliterated strial vessels.) The spiral ligament appeared normal. The *organ of Corti* was well-preserved. Cell counts revealed a prominent loss of hair cells at the apex and slight loss at the base. The ganglion cell population was relatively decreased at the apical and mid-portions of the spiral ganglion (Figs. 1, 2).

Saccule. The extremely dilated saccule abutted the stapes footplate and the dilated wall caused a layered arrangement of the epithelial walls at the junction of the saccule, utriculo-saccular duct, and utriculo-endolymphatic valve. An epithelium-covered papillary mound from the saccular wall was present, extending into the lumen of the sacculus. This epithelial covering appeared continuous with



Fig 1 Midmodiolar section, left ear showing Reissner's membrane herniation through helicotrema, organ of Corti, ganglion cell content of modiolus and complete absence of apical sensorineural epithelial structures.

the anterior and posterior portions of the adjacent membranous wall (Fig. 3) This was similar in appearance to a healed rupture (rent) mechanically produced in the cat (Fig. 3). The macula and otoliths were normal in appearance except for scattered vacuolated sensori-epithelial cells.

Utricle and semicircular canals The utricular wall was dilated and irregular The irregularity was caused by multiple herniations (Fig. 4) of the membranous walls extending into the ampullary ends of the semicircular canals. This arrangement resulted in a scalloped configuration of the membranous ampullary walls opposite all cristae (Fig. 4). A herniated portion of the utricle extended into the common crus. An epithelial-covered mound smaller but similar to that present in the sacculus, and a fibrinous joined membranous break were seen near the medial arm of the horizontal canal. The otoliths and macular membrane appeared normal as did the sensorineural epithelium of the cristae.

Endolymphatic duct and sac No abnormalities were seen. Normal vascularity was present throughout (Fig 5)

Right Temporal Bone

(Bubble formation caused some processing artifact which was taken into consideration during evaluation.)

The findings of the right temporal bone were similar to the left. In the *cochlea* there was marked dilatation of Reissner's membrane and strial atrophy There was prominent ex

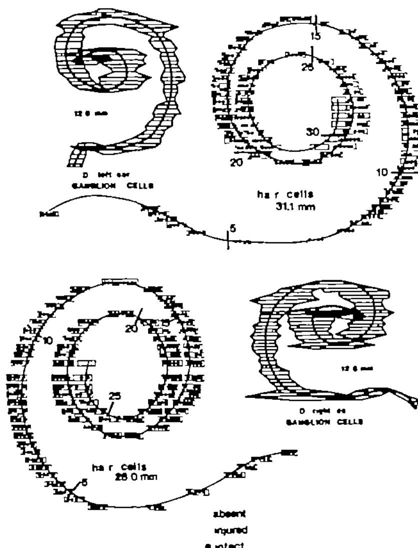


Fig. 2. Graphic reconstruction of the organs of Corti and ganglion cell populations. Compare the density of the ganglion cell populations with that of a normal ear in Fig. 6. Note the loss of hair cell population in the apical turn and the ganglion cell loss in the apical and mid-portions of the modiolus.

ternal hair cell loss at the apex. The ganglion cell population was decreased in the mid and apical portions. The *saccule* showed dilatation, membranous wall collapse with healing to the otolith-deficient macula, and polypoid mass formation. The *utricle* and *semicircular canals* showed dilatation and ampullary wall scalloping. No abnormalities were seen in the *endolymphatic duct* and *sac*.

DISCUSSION

Altmann & Kornfeld (1965) summarized the reported changes of membranous labyrinthine hydrops in cases of Menière's disease as follows:

(1) Simple dilatation.
(2) Dilatation of endolymphatic structures with extension into spaces normally not occupied by them.
(3) Circumscribed outpouchings with the walls having the same appearance as the remainder of the structure from which they are derived.
(4) Circumscribed thin wall outpouchings.
(5) Ruptures of the walls.
(6) Collapse of the walls.

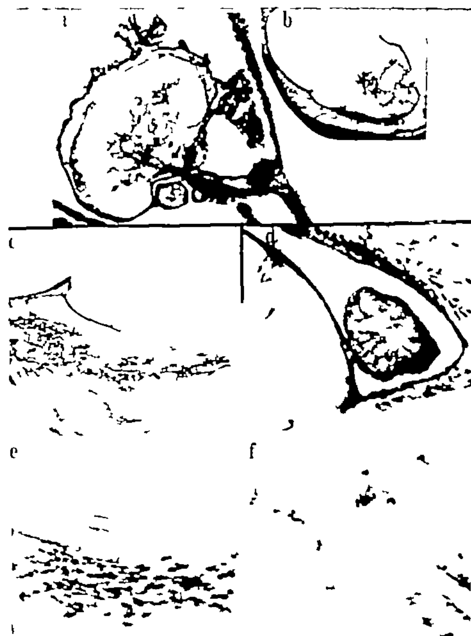


Fig 3 Evidence of rupture and healing of membranous labyrinthine wall. ( ) Left ear High power view of the papillary lesion of the saccular wall (*b*) Left ear Low power view of (*a*) for orientation. (*c*) Right ear Section through the saccular wall showing collapse and healing to the macula. (*d*) Right ear Papillary growth adjacent to the reinforced area shows double membrane cellular closure (*e*) Right ear High power view of the area of healing of the saccular wall to the macula (*f*) Cat specimen. Healed papillary lesion of the saccular wall and macula mechanically produced in the cat.

They indicated, as had others (Schuknecht et al., 1962), the presence of tissue changes about the cristae due to edema of the connective tissue or mechanical displacement These areas which had been indicated as areas of membranous rupture by some (Hallpike & Cairns, 1938 Schuknecht et al., 1962) were interpreted by Altmann & Kornfeld (1965) as simple thin wall dilatations.

Demonstration of rupture was considered rare by Altmann & Kornfeld (1965). Those cases showing curling of defect borders were

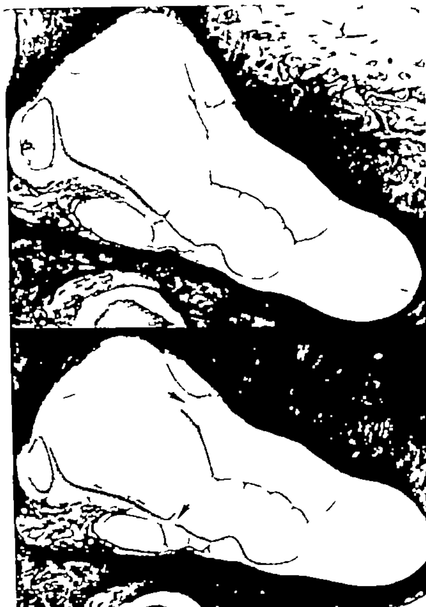


Fig 4 Left ear Two sections 80 μ apart at level of horizontal canal cristae *showing infolding of utricular membranes* (arrows at neck of herniations) resulting in double-walled membranes, to be distinguished from break in membranous labyrinthine wall (ruptures). Note scalloped configuration of the membranous ampullary wall.

not accepted as histologic evidence of rupture during life. Multiple defects of this type found in a single specimen, they proposed, could be explained only on the basis of artifact, since distention could cause but one rupture which on occurring would release that pressure necessary for further membranous breaks. The previous proposal of circumscribed pouches as evidence for healed rupture (Schuknecht et al., 1962) was dismissed because of the uniform regularity of the wall They interpreted connective tissue strands in the scala vestibuli previously given as evidence of rupture (Hallpike & Cairns, 1938 Lawrence & McCabe 1959) as a consequence of periosteal irritation They stated that evidence of connective tissue proliferation and scar formation were necessary for documentation of membranous rupture.

Schuknecht (1968) described a polypoid

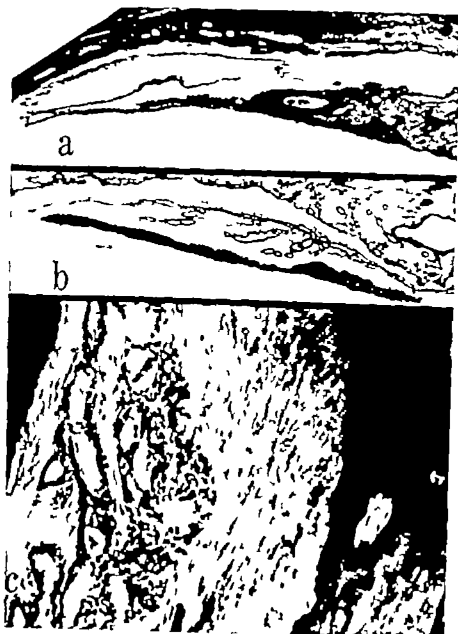


Fig. 5. (*a*, *b*) Left ear endolymphatic duct and sac; (*c*) Right ear. High power view of endolymphatic sac. Note adequate vascularity. Many vessels are empty of blood elements but are clearly distinguishable.

mass arising from the saccular wall in a recent report. He stated that it was choroid in appearance but did not attribute any function or significance to its presence. Similar masses were seen in the case of idiopathic hydrops presented (Fig. 3). The masses appear to be an epithelial overgrowth of the wall of the saccule having an irregular connective tissue stroma. A similar finding was present in a cat's saccular wall ruptured for vestibular experimentation purposes (Fig. 3). Although these are not identical in appearance, we propose that these lesions are evidence of healed rupture fulfilling the requirements of Altmann & Kornfeld (1965).

That marked dilatation of the entire endolymphatic system could occur in labyrinthine hydrops was fully demonstrated by the specimen described. Multiple dilatations of the utricle with sac-like formations (Fig. 4) and thin-walled para cristae herniations were present. Scalloped distortions of the ampullary wall were distinct. No evidence of rupture healing was observed in these areas. Reisner's

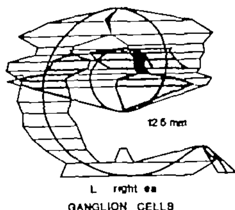


Fig 6. A graphic representation of the number and spatial location of the ganglion cell population in an ear with normal audiometric responses. The heavily shaded areas represent double and triple overlapping of the ganglion cell population when represented in two dimensions. Compare the abundance of cells in the second turn with relative smaller number shown in Fig. 2.

membrane was dilated throughout, herniating through the helicotrema into the scala vestibuli.

The suggestions in the literature (Shambaugh, 1967) that hydrops of the membranous labyrinth could be related to compromised vascularity about the endolymphatic duct, were not supported by the findings in these temporal bones (Fig. 5) A normal appearing periductal vascular system was present.

In this case of idiopathic hydrops, cell counts showed loss of hair cells which was most pronounced in the apex of each ear Some areas demonstrated a total absence of the organ of Corti (Fig. 1) The ganglion cells were counted and reconstructed graphically* for each level and area of the cochlea (Fig. 2) This allowed visual comparisons with the normal (Fig. 6) demonstrating the relative loss and location of loss of ganglion cell population.

**Graphic representation of ganglion cell population.* The method used for mapping the ganghon cell spatial relationships was similar to the cochleogram methods described by Guild et al. (1931) and Crowe et al (1933) and ganglion cell methods of Schuknecht (1953) Those serial sections in which the modiolus was represented were considered in sequence. The first ganglion cell encountered in any turn was considered equivalent to the tangentially cut juncture of the pillar cells in cochleograms, the last cell of the opposite side of the spiral turn equivalent to its associated tangentially cut juncture of the pillar cells. These points were plotted (4 in number) on the vertical axis, in the same manner using the same scale as the cochleogram. Semi-circles were used to connect the points as in hair cell mapping.

The grouping or bunching of ganglion cells (Crowe et al., 1933) was observed and noted during the cell count. Three areas of bunching occurred (central in the modiolus, lateral and medial) for each turn. The number of cells were recorded for each 100–200 μ (5–10 sections) The number of cells for each group at each level counted was represented by a line proportional in length to the number of cells times an average cell size, (i.e., 50 cells × 15 μ × scale = length of line). This method accounted for the overlapping seen on the two-dimensional graphs but allowed visual representation of the pattern of cell distribution.

Observations of hair cell loss in cases of labyrinthine hydrops have been irregular in the literature. Since the report of hair cells loss by Hallpike & Cairns (1938) six cases have demonstrated hair cell loss (Day & Lindsay 1949 Kristensen, 1961 Lindsay & Schulthess, 1958 Lindsay et al., 1967 Nager 1949) not attributed to autolysis. One of these (Kristensen, 1961) appeared to be compatible with processing artifact when the published photographs were reviewed. Three of the other cases were complicated by otic inflammation. Estimations of ganglion cell loss have been reported four times (Altmann & Fowler 1943 Lindsay 1944 Lindsay & Schulthess, 1958 Lindsay et al., 1967) Other papers, quantitatively evaluating the hair cell population, demonstrated no loss of hair cells and reported no loss of ganglion cell population, or demonstrated a loss for the highest frequencies only (Schuknecht, 1962, 1968).



Fig. 5 (*a*, *b*) Left ear endolymphatic duct and sac. (c) Right ear. High power view of endolymphatic sac. Note adequate vascularity. Many vessels are empty of blood elements but are clearly distinguishable.

mass arising from the saccular wall in a recent report. He stated that it was choroid in appearance but did not attribute any function or significance to its presence. Similar masses were seen in the case of idiopathic hydrops presented (Fig. 3). The masses appear to be an epithelial overgrowth of the wall of the saccule having an irregular connective tissue stroma. A similar finding was present in a cat's saccular wall ruptured for vestibular experimentation purposes (Fig. 3). Although these are not identical in appearance, we propose that these lesions are evidence of healed rupture fulfilling the requirements of Altmann & Kornfeld (1965).

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have resulted in erroneous information to the higher centers. As an ongoing process, dilatation and distortion could have resulted in vertigo of various durations depending on the degree and duration of the process. Observations during acute attacks of vertigo of a changing vestibular response with wide variations would be compatible with this proposal. The periods of quiescence between vertiginous attacks may well have been related to a stationary configuration of the membranous walls, the still altered, but not actively changing, cupular response accommodated by some process similar to habituation.

On the other hand, ruptures causing endolymph to be contaminated by perilymph have been cited as the cause of vertigo (Schuknecht et al., 1962). Changes seen in some temporal bone specimens have been interpreted as the result of an *in vivo* process. These interpretations were challenged, because of the lack of firm evidence of healing mechanisms in those areas of break of the membranous walls. The recent observations of a polypoid mass of the saccular wall in two patients with hydrops and in the utricle in one patient suggested the presence of a healing process. A similar finding in an animal specimen with known sacculus rupture supported this interpretation. That rupture alone has been the cause for the vertiginous episodes has not been supported in the greater number of histologic observations. Further the paralytic nystagmus (nystagmus in one direction only with the quick component away from the perfused ear) seen in animal experimentation with perfusion of the perilymphatic space with potassium rich solution (Silverstein, 1970), is neither in agreement with the nystagmus duration nor the direction changing nystagmus seen in acute Menière's attacks (Mygind & Dederding, 1938). Even though the phenomenon of functional disturbances due to ampullary distortion has histologic support, the possibility exists that rupture may serve as an additional mechanical, or as a chemical, factor.

The phenomenon of hearing fluctuation and loss was less easily explained. Two types of histologic observations have been reported in addition to the uniformly present dilatation of the scala media. In those quantitatively evaluated specimens from patients with cochlear hydrops, findings of the loss of neuro-epithelial elements have conflicted with the more regular finding of intact hair cells and ganglion cells. If both sets of observations have been correct then both should have correlated with the clinical pictures of fluctuating and permanent hearing loss.

The mechanism for fluctuating hearing has not been explained by either set of observations. Earlier proposals to attribute the early auditory findings to inner ear conduction impairment have failed to explain the progressive sensorineural impairment (Lindsay 1942, 1946 Mygind & Dederding 1938). Proposals attributing fluctuating hearing to alterations of the membranous labyrinth by distention on one hand, and rupture with endolymph contamination by perilymph on the other have been made. These mechanisms would presumably be of two types, one involving a homogenous endolymphatic composition and the other localized contamination of endolymph by perilymph. These two views remain unresolved.

The findings of loss of ganglion cells and hair cells in but a few appropriately evaluated specimens of labyrinthine hydrops may well have represented an older stage of the disease process. It would be difficult to attribute the loss of ganglion cells in the modiolus to the mechanical effect of scala media dilatation. It has been suggested that some form of metabolic abnormality could explain the changes in cochlear function, and the reported variations in cellular loss. Recent experimental information has shown cortilymph to be identical to both perilymph, and the fluid within the modiolus. Open communication from the scala tympani to the modiolus to the tunnel of Corti and the basal portion of the hair cells exists allowing the bathing of these elements in perilymph (Fernández, 1951 Hinojosa, 1969; Ilberg & Vosteen, 1969). This would sug-

gest that a perilymphatic metabolic change in labyrinthine hydrops could account for the changes seen in both the hair cells and ganglion cells. Likewise, these metabolic processes could allow fluctuating function without loss of structure as in the greater number of histologic evaluations. We would suggest that the dilatation of the scala media and the perilymphatic metabolic effects are two separate phenomena, probably related, but only the latter accounting for the hearing changes in hydrops. In this specimen the loss of both ganglion cells and hair cells, would be in agreement with a metabolic process.

ZUSAMMENFASSUNG

Zwei Schläfenknochen wurden beschrieben, die bilaterale Labyrinthine Hydrops, verbunden mit einer Degeneration des Corti'schen Organs und den Verlust von Ganglienzellen, demonstrieren. Erweiterungen mit sack artigen Ausstreuungen der utricularen Wand waren auffällig. Der Sacculus zeigte gleichzeitig Erweiterung und teilweisen Zusammenfall. Veränderungen beiderseits in den Sacculi und in einem Utriculus wurden als Heilung einer Zerreissung gedeutet. Funktionelle und pathogenetische Bedeutung dieser Befunde wurden diskutiert.

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Acta Otolaryng 73 413–417 1972

RESPONSES FROM ISOLATED INDIVIDUAL SEMICIRCULAR CANAL AMPULLAE IN FROG

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(Received June 9 1971)

Abstract By using isolated semicircular canals of frogs and a stimulator which induced a current of fluid within the ampulla the author proved the correctness of Ewald's law on the basis of the responses from the ampullar nerve. Namely in the anterior and posterior canals an ampullofugal current showed a stimulating effect, while in the lateral canal an ampullopetal current provoked an increase in the nerve discharge. On alternate ampullofugal and ampullopetal currents given from the site of the utricular duct through the lateral and anterior canals, an alternating increase in the nerve discharge was observed, as the form of receptor function is opposite in these canals. The present method is believed to be a unique way to study Ewald's law

Ewald (1892) a German physiologist, stimulated the labyrinth of pigeons with the famous pneumatic hammer to study movement of the head, and concluded that in the lateral semicircular canal an ampullopetal movement of endolymph had a greater effect than ampullofugal movement, whereas in the anterior and posterior canals the latter had a greater effect. This fact was introduced in the clinical study of the labyrinth by Bárány at the beginning of this century and was called the second law of Ewald. Later Löwenstein & Sand (1940) and Groen et al. (1952) made electrophysiological experiments and proved its correctness. However there is still some argument as to whether this law holds true in every animal.

The author stimulated the isolated semicircular canals of frogs with a device which induced ampullofugal and ampullopetal currents in each semicircular canal with evoked action potentials in each of the ampullar nerves so that Ewald's laws could be studied.

MATERIAL AND METHOD

The heads of frogs (*Rana nigromaculata*) were amputated and cut into halves in the sagittal plane. Bone and cartilage covering the labyrinth soaked in Tyrode solution were removed with a watchmakers pincetto from the palatal side under control of a preparation microscope. The saccule was visualized and appeared whitish because of the otoliths; the VIII nerve was cut at the entrance into the brain brain and medulla were removed, on removal of the bony wall in front of the brain the posterior semicircular canal became apparent, the canal end of which was cut first, and next posterior ampullar nerve from the vestibular nerve trunk, then the ampullar end was cut. The posterior semicircular canal was now isolated. By amputation of the canal end of the lateral and anterior semicircular canals these canals, with the saccule attached, were easily isolated. As the lateral and anterior semicircular canals were connected to the utricle with the utricular duct, the two canals could be isolated by cutting the utricular duct.

Stimulation was induced by use of a device (see Fig. 1) (as previously reported, see Harada et al., 1966, 1967 Harada, 1970) to as-

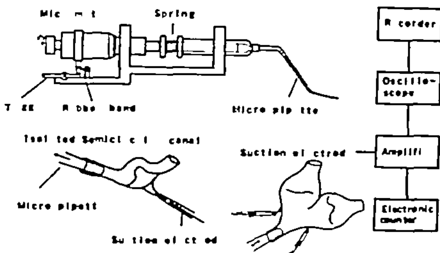


Fig 1

pirate or inject 0.15μl of the fluid into the posterior semicircular canal via a micropipette inserted into the canal end.

In the lateral and anterior semicircular canals the micropipette was inserted into the utricular duct, as depicted in Fig. 1 The action potentials from the ampullar nerves were detected with a glass micro suction electrode consisting of two platinum wires, internal and external.

The ampullar branches from the lateral and anterior semicircular canals unite to form a nerve trunk which enters the vestibular nerve. The nerve trunk was cut at the junction with the vestibular nerve and divided into the two ampullar branches. Detection of potential through two suction electrodes is shown in Fig. 1 Amplification through a biophysiological amplifier observation on an oscilloscope and recording by a continuous recording camera were obtained.

RESULTS

The action potentials in the isolated posterior ampullar receptors

On aspirating fluid from the ampulla through the canal end of the posterior semicircular canal with the stimulating device an ampullofugal current was produced in the canal, which resulted in a marked increase in the frequency of discharges from the ampullar nerve (see Fig 2) The number of spikes over 50 μV amplitude in a 7 sec period was determined by electronic counter to be about 1 500 (Harada et al., 1966). However an increase in the number of spikes was not observed when an ampullopetal fluid current was induced by injection in the ampulla.

Fig. 3 shows the potential obtained through a d.c. amplifier on similar ampullofugal and ampullopetal currents. When an ampullofugal current was induced, a rapid rate of rise in the

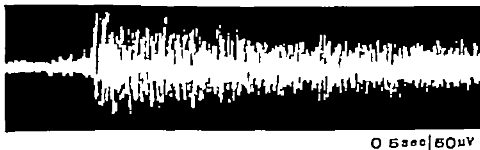


Fig Response from the posterior ampullar receptor

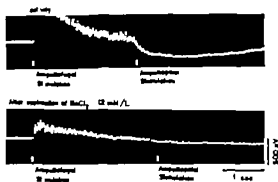


Fig. 3. Slow potential from the posterior ampullar receptor.

slow potential of 400–600 μV was recognized and the number of spikes was increased. When an ampullopetal fluid current in the ampulla was induced by an injection stimulus at the point of return of the positive slow potential to the base line, a negative slow potential appeared with a decrease in the frequency of nerve impulses. Since these positive and negative slow potentials were inhibited by Ba^{2+} ion (Harada, 1971) it is concluded that these potentials were not an artifact but a true biologic potential.

From these experiments it was concluded that in the posterior semicircular canal an ampullofugal flow has a stimulating action while an ampullopetal flow has a rather inhibitory action on the receptors.

Action potentials of isolated lateral and anterior semicircular canal receptors

The action potentials from each ampullar nerve were detected simultaneously to study how the ampullofugal and ampullopetal fluid currents acted on the receptors of each semicircular canal.

When an aspiration or injection is made simultaneously on the two ampullae from the utricular duct, it would be expected (as is learned from the Ewald's law) that on aspiration the lateral and on injection the anterior semicircular canal is stimulated.

As seen in Fig. 4 in the lateral semicircular canal the frequency of nerve discharges was increased by the ampullopetal current, while in the anterior canal the spontaneous discharge was decreased. On the contrary the inactive injected ampullofugal current caused an increase in the number of nerve discharges from the anterior canal, while the spikes from the ampullar nerve of the lateral canal did not increase.

It was now concluded that in the isolated anterior and posterior semicircular canals of frogs an ampullofugal current served as stimulus, whereas in the lateral canal an ampullopetal current served as stimulus, resulting in increases in the frequency of nerve impulses. In other words, Ewald's law was proved to be correct by observing the action potentials in response to mechanically induced flow of fluid within each of the three isolated semicircular canals.

DISCUSSION

Ewald's second law indicates that in the horizontal semicircular canal an ampullopetal lymph current causes a stronger reaction than an ampullofugal current and the reverse direction serves as a stimulus to the anterior and posterior canals. According to Ewald the crista ampullaris has a "labyrinthine tone" a constant muscular tone which is maintained by the labyrinthine organs during rest. He stated that in the lateral canal an ampullopetal current increased this tone and an ampullo-fugal current reduced the tone.

Concerning this fact Löwenstein & Sand (1940) and Ross (1936) reported in support of this law on fish and frogs, and on the fitness of the law in mammalia, Gernandt (1949) and Eckel (1954) also made reports. With respect to the morphology Wersäll (1961) and Flock (1965) performed electron microscopic studies on the crista ampullaris revealing that the sensory hairs consisted of a single kinocilium and many stereocilia. Further it was shown electrophysiologically that a depolarization took place when the stereocilia were inclined to the kinocilium

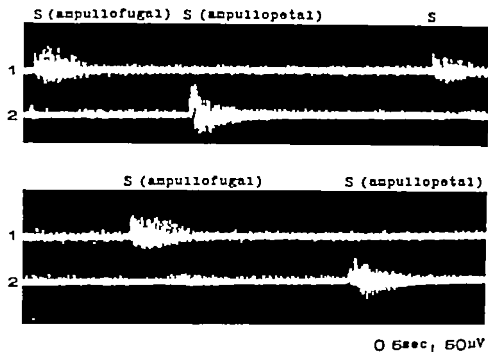


Fig. 4. Responses from the anterior canal and the lateral canal. *1.* anterior canal, *2.* lateral canal. *S.* stimulation.

and in the opposite case a hyperpolarization occurred. It was also found that the kinocilium lay on the side of utricle in the lateral canal and on the side of the semicircular canal in the anterior and posterior canals.

The author has reported experiments using the isolated posterior semicircular canal of frogs in which the nerve impulses on stimulation with an ampullofugal current were counted with an electronic counter and the relation between the concentration of some ototoxic agents and the resulting decrease in number of spikes was tested (Harada et al. 1966, 1967, Harada 1970).

In the present work the author intended to prove Ewald's law. The anterior and horizontal canals were isolated. When the fluid in the two ampullae is aspirated or injected through the utricular duct an ampullopetal flow occurred with an increase in nerve discharges from the lateral canal as seen in Fig. 5 and on injection an ampullofugal current with an increase in nerve discharges from the anterior canal.

Analysis of the positive and negative slow potentials from the posterior canal obtained through a d.c. amplifier showed that in the posterior canal an ampullofugal flow had a stimulating while an ampullopetal flow had an inhibiting action.

McNally & Tait (1925) and Maxwell (1923) found that in frogs the reaction was gained only in response to a certain direction of current of the endolymph within the semicircular canal. However, it is known that in human canals flow in either direction can be an effective stimulus. Groen (1961) studied the action current in response to rotating stimulation using a three-fibre preparation from the horizontal ampullar nerve of rays. He reported that by rotation in the direction of stimulation the frequency of the spontaneous discharge increased and by rotation in the opposite direction it decreased, and the response, expressed in the number of action potentials per second as a function of the stimulus, appeared to have an S-shape like a triode characteristic with a straight part between −40

and −40 /sec. Ledoux (1958) reported that in frogs a similar suppressive mechanism could be observed in response to negative stimuli but the response was smaller than in rays. Löwenstein & Sand (1940) found a resting activity in elasmobranch labyrinth which they believed to give muscles a constant tonus.

In fact, even from isolated semicircular canal receptors a spontaneous discharge can be obtained. In the anterior and posterior canals a marked increase in the nerve discharge is seen on an ampullofugal current. With an ampullopetal current a negative slow potential appears in recordings with a d.c. amplifier but the potential is smaller than that gained with an ampullofugal current. Although some decrease in the nerve discharge was observed, the S-shape reported by Groen et al. (1952) was not distinct. This fact appears to agree rather well with the report of Ledoux (1958). In the lateral canal an ampullopetal current has, as stated above, a stimulating effect.

The present method of testing the function of each individual semicircular canal without influence of centrifugal nerve fibers, seems to be very unique and Ewald's second law was thus proved to be correct by electrophysiological experiments with isolated semicircular canals of frogs.

ZUSAMMENFASSUNG

Isolierte Bogengänge des Frosches wurden mittels eines Gerätes gereizt, welches experimentell eine Strömung der Flüssigkeit in der Ampulle erzeugen konnte. Die vom nervus ampullaris gewonnenen Reaktionen sprachen für das Ewald'sche Gesetz. In den vorderen und hinteren Bogengängen wirkte eine ampullofugale Strömung als effektiver Reiz, eine Vermehrung der Nervenentladung herbeizuführen, während es im horizontalen Bogengang eine ampullopetale Strömung war. Bei einer gleichzeitigen Reizung der horizontalen und vorderen Bogengänge, in welchen die Formalität des Rezeptorenmechanismus umgekehrt ist, vom ductus utriculus her mit ampullofugaler bzw. -petaler Strömung, liess sich eine abwechselnde Vermehrung der Nervenentladung erkennen.

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Acta Otolaryng 73 418–427 1972

SQUAMOUS CELL CARCINOMAS OF THE PALATE

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(Received July 1, 1971)

Abstract A histological survey of 383 primary tumours in the palate revealed that 219 were malignant tumours, 123 of which were squamous cell carcinomas (56%) and 75 malignant salivary gland tumours (34%). The squamous cell carcinomas were classified as either highly differentiated (83 cases) or poorly differentiated (40 cases) and the morphological basis for distinguishing the two types has been discussed. The investigation revealed that the poorly differentiated carcinomas were more commonly ulcerated and had a higher incidence of metastases and lower survival rate than the highly differentiated carcinomas.

A comparison was made between different clinical features of the two predominant groups of malignant tumours of the palate—squamous cell carcinomas [illegible] malignant salivary gland tumours. It was found [illegible] squamous cell carcinomas are more commonly [illegible]ized to the soft palate, are generally larger in [illegible] and more often ulcerated than malignant salivary gland tumours. Squamous cell carcinomas have also a shorter period of symptoms before therapy but despite this the prognosis is worse than for malignant salivary gland tumours.

PRESENT SERIES

The present study is based on a histological survey of tumours of the palate in 383 patients registered at Radiumhemmet and the Department of Otolaryngology Karolinska Sjukhuset, during the period 1909–1966 Of the total number 219 (57%) were malignant 75 salivary gland tumours, 123 squamous cell carcinomas, 13 mesenchymal tumours and 8 melanomas. A histological re-examination was possible of all squamous cell carcinomas, since the tumour material is available at the Department of Tumour Pathology A histological-clinical correlation study was made in 112 patients who could be followed-up for more than 5 years and none of whom were lost to follow-up The prognosis was investigated by studying the rate of local recurrence, metastases and the determinate survival rate, which is based upon determinate groups (Eneroth et al., 1970). These do not include patients who have died of intercurrent disease. The follow-up period ranged from 5–39 years.

HISTOLOGICAL FEATURES

The 123 cases of squamous cell carcinomas of the palate were all invasive but exhibited a considerable variation in differentiation, degree of keratinization and cellular structure. Numerous tumours showed exophytic papillomatous growth at the surface and no less than 90 (73%) had ulcerated the mucosa. In order to find out whether prognostic differences exist between highly and poorly differentiated tumours, the tumours were registered in one of these two groups on reclassification. In Figs. 1–3 examples are given of tumours classified as highly differentiated. Thus tumours with well defined cords and strands of epithelial cells, as well as such tumours as showed obvious keratinization, were recognized as highly differentiated. In this respect little attention was paid to the degree of cellular polymorphism. Tumours with diffuse growth, little or no

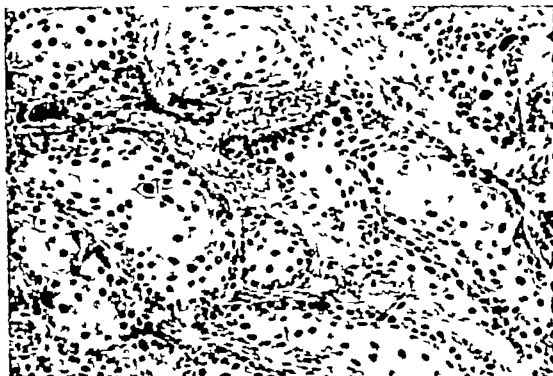


Fig 1 Highly differentiated squamous cell carcinoma. Well defined cords. Moderate cellular polymorphism. Slight keratinization. Photomicrograph, × 160.



Fig 2 Highly differentiated squamous cell carcinoma. Well defined cords. Moderate cellular polymorphism. No keratinization. Photomicrograph, × 160.

Fig 3 Highly differentiated squamous cell carcinoma. Well defined cords. Considerable cellular polymorphism. Slight keratinization. Photomicrograph, 160.

Fig 4 Poorly differentiated squamous cell carcinoma. Fairly well defined cords. Marked cellular polymorphism. No keratinization. Photomicrograph, 160.

Fig. 5. Poorly differentiated squamous cell carcinoma. Diffuse growth. Marked cellular polymorphism. No keratinization. Photomicrograph, 160.

Fig. 6. Squamous cell carcinoma. Highly and poorly differentiated components. Photomicrograph, 160.



Fig. 7 Squamous cell carcinoma. Highly differentiated tumour with invasive growth. Little cellular polymorphism. Photomicrograph, × 160.

dency to form cords, and showing no sign of keratinization (Figs. 4–5) were diagnosed as poorly differentiated carcinomas.

One problem in the classification was that in some cases the carcinomas showed a highly differentiated structure at the surface and a diffuse growth corresponding to a poorly differentiated carcinoma in the deeper portions (Fig. 6). Another problem was that highly differentiated tumours growing in small disseminated cords showed so little polymorphism that it might be questioned whether they were true carcinomas (Fig. 7). In these cases metastases revealed the malignant nature of the neoplasms.

In the histological survey of the 123 tumours 83 were registered as highly differentiated carcinomas and the remaining 40 accordingly as poorly differentiated. There was no obvious difference between the two types as far as depth of growth or stromal reaction was concerned. The poorly differentiated carcinomas were more commonly ulcerated than the highly differentiated (see below).

CLINICAL FEATURES

Highly differentiated squamous cell carcinoma

Of the 83 patients with this type of tumour 49 (59%) were men and 34 (41%) women. The age of the patients ranged from 33–84 years (mean 62.2 years). The interval between appearance of the first symptoms of the tumour and therapy was less than one year in 76 of the patients. In 6 of the remaining 7 patients the interval was 1 to 3 years and in one case the interval was more than 3 years. Thirty-six

of the patients noticed a lump in the palate. Pain, ulceration and bleeding were observed by 22, 23 and 6 patients respectively. Three patients complained of difficulties in wearing their dentures. In 32 cases the tumours were localized to the hard palate, in 38 to the soft palate, and in 13 cases to both the hard and soft palate. Extension beyond the palate was observed in 32 cases, most commonly to the tonsils, the nasal cavity and the maxillary gingiva. In many of these cases the tumours were very advanced and presented a diffuse extension. The tumours had an approximate diameter of less than 2 cm in 20 cases, between 2 and 4 cm in 32 cases, and more than 4 cm in 31 cases. The surface of the tumours was stated to be ulcerated in 55 of the 83 cases (66%)

Poorly differentiated squamous cell carcinoma

Twenty-two (55%) of the 40 patients with poorly differentiated carcinomas were men. The age of the patients ranged from 34–85 years, the mean age being 64.5 years. The duration of the symptoms before therapy was less than 1 year in 39 cases and 1.5 years in one case. A lump in the palate was noticed by 13 patients and pain, ulceration and bleeding by 9, 19 and 2 patients respectively. In 16 cases the tumour was localized to the hard palate, in 21 cases to the soft palate, and in 3 cases to both the hard and soft palate. Extension beyond the palate was noticed in 15 cases, in most of them to the tonsils and nasal cavity. A great number of these poorly differentiated carcinomas were very advanced and diffusely circumscribed. The approximate diameter of the tumour was less than 2 cm in 11 cases, between 2 and 4 cm in 13 cases, and more than 4 cm in 16 cases. The surface of the tumours was stated to be ulcerated in 35 of the 40 cases (88%)

TREATMENT

The treatment of the squamous cell carcinomas of the palate has been surgery or irradiation, often in combination. The principles of therapy have, however, varied during the long period covered by the tumour material.

The surgical treatment consisted of extirpation of the tumour in 26 cases, combined with resection of the bony palate in 10 and with electrocoagulation in 7 cases. In 12 cases the surgical procedure was denoted only as electrocoagulation. In no less than 85 cases was the surgical procedure limited to excision with small margin or to mere biopsy. Irradiation was administered in 114 of the 123 cases.

PROGNOSIS

The prognosis of the squamous cell carcinomas of the palate is based on a clinical follow-up study of 112 patients, 75 with highly differentiated and 37 with poorly differentiated tumours. The observation period ranged from a minimum of 5 years to 39 years. No patient was lost to follow-up. The evaluation of the prognosis was based on local recurrences, metastases and survival rates.

Recurrence

In the 75 cases with highly differentiated carcinoma, a recurrence arose within 1 year in 7 cases and in another 4 cases within 1, 2, 4 and 7 years, respectively after treatment. In the 37 patients with a poorly differentiated carcinoma a local recurrence was found in 5 cases, all within 1 year of treatment.

Metastases

Metastases were demonstrated in 27 of the 75 patients (36%) with a highly differentiated carcinoma. In 16 of the 27 cases metastases were present already at the first examination. In the other 11 cases the metastases appeared later during the follow-up period. In 25 cases metastases were localized to the regional lymph nodes and in 2 cases to the lungs. In no case with highly differentiated carcinoma lymph node and distant metastases were found

Table I Squamous cell carcinoma of the palate 5–39 years' follow-up study of 112 patients

Histological features	No. of patients	Died of: Tumour disease	Died of: Intercurrent disease
Highly differentiated	75	38 (51 %)	29
Poorly differentiated	37	25 (68 %)	12
Total	112	63 (56 %)	41

simultaneously In 18 of the 25 patients with lymph node metastases the interval between the first symptoms of the tumour and the demonstration of metastases was less than 1 year In the 2 cases with distant metastases the interval was between 1 and 2 years.

Metastases appeared in 20 of the 37 patients (54%) with a poorly differentiated carcinoma. In 11 of the 20 cases metastases were present already at the first examination. In the other 9 cases metastases appeared during the follow up period. In 5 patients there were both regional lymph node metastases and distant metastases. In the remaining 15 patients only regional lymph node metastases were found. Distant metastases appeared in the lungs, the skeleton, the liver the kidney and the spleen. In 3 cases distant metastases were present at more than one site. The interval between the first symptom of the tumour and the demonstration of metastases was short, in 17 of the 20 cases with lymph node metastases less than 1 year In 4 of the 5 cases with distant metastases the interval was between one and two years

Survival

The death rates of the 112 patients with squamous cell carcinoma of the palate are demonstrated in Table I. During the follow-up period 63 patients (56%) had died from their tumour disease Of the patients with highly differentiated carcinomas 51% and of those with poorly differentiated types of tumour 68% had died of the tumour disease. Eight patients with a highly differentiated tumour were alive at the last follow-up examina-

II *Highly differentiated squamous cell carcinoma of the palate 5–20 years follow-up study 75 patients*

Observation period (years)	No of patients	Died of: Tumour disease	Died of: Intercurrent disease	Determinate survival rate: No of patients	Determinate survival rate: No. of survivors	Determinate survival rate: Survival (%)
5	73	36	6	69	33	48
10	67	36	12	55	19	35
15	57	31	16	41	10	24
20	51	29	18	33	4	12

Table III *Poorly differentiated squamous cell carcinoma of the palate 5–20 years follow-up study of 37 patients*

Observation period (years)	No. of patients	Died of: Tumour disease	Died of: Intercurrent disease	Determinate survival rate: No. of patients	Determinate survival rate: No. of survivors	Determinate survival rate: Survival (%)
5	37	25	4	33	8	24
10	33	21	7	26	5	19
15	29	18	8	21	3	14
20	19	13	4	15	2	13

with a mean of 14 years survival after first treatment. All patients with a poorly differentiated tumour died during the follow period. The number of patients who died intercurrent disease is admittedly large, it must be pointed out that the average age the patients with squamous cell carcinoma as 63 years at the first treatment of the tu-

The determinate survival rates (D.S.R.) af 5 10, 15 and 20 years' observation in the of patients with highly differentiated arcinomas are demonstrated in Table II The corresponding figures are given for the patients with poorly differentiated carcinomas in III. As can be seen from the tables, the D.S.R. for highly differentiated carcinomas decreased from 48% in the 5-year group to 12% in the 20-year group. The corresponding figures for poorly differentiated carcinomas were 24 and 13% respectively The 5-year D.S.R. for all 112 patients with squamous cell carcinomas was 41% and the correspond figures for 10, 15 and 20 years were 30% and 13% respectively

DISCUSSION

us cell carcinoma is the most com type of malignant neoplasm of the palate. The relative incidence of squamous cell carcinomas varies, however greatly in different Thus Martin (1942) in a material of palatal tumours from Memorial Hospital, New York, found that 83% of the malignant rs were squamous cell carcinomas and ohn (1961) reported 70% squamous cell out of 65 malignant palatal tu rs collected at the Mayo Clinic (surveyed by Dahlin, 1968). In the present study based upon 383 cases of primary tumours in the palate, 219 were found to be malignant, 123 of which were registered as squamous cell carcinoma (56%) The majority of the remaining malignant tumours were of salivary land origin. Since squamous cell carcinomas salivary gland carcinomas are thus the most common types of malignant tumours of the palate a comparison between the clinical appearance of these two tumour types is of interest.

Squamous cell carcinomas are slightly more common in men than in women (71 out of 123), whereas no such differences is found for salivary gland carcinomas. The mean age for the occurrence of squamous cell carcinomas, furthermore, is somewhat higher (mean age 63 years) than for salivary gland carcinomas (less than 60 years). No less than 94% of the cases of squamous cell carcinomas had had symptoms for less than 1 year In all cases with poorly differentiated muco-epidermoid carcinomas the interval between first symptom and therapy was less than 1 year (Eneroth et al., 1970). For the remaining types of salivary gland carcinomas, however the period of symptoms was generally longer (38% more than 1 year) Squamous cell carcinomas appeared as a lump in 40% of the cases, where as this was the first symptom in more than 80% of the salivary gland carcinomas. On the other hand no less than 73% of the squamous cell carcinomas (66% and 88% of the highly differentiated and poorly differentiated, respectively) were ulcerated, but only 30% of the salivary gland carcinomas. There was also a difference in the primary location of the two tumour types. Thus a majority of the squamous cell carcinomas developed in the soft palate (75 out of 123), whereas salivary gland carcinomas were more commonly found in the hard palate (only 21 out of 75 cases in the soft palate according to Hjertman et al., 1970) This finding is in agreement with earlier reports (e.g. Martin, 1942). Extension of the tumours beyond the palate was as common in cases of squamous cell carcinoma as in those with salivary gland carcinoma. The latter type of tumour was also generally smaller in size at diagnosis than squamous cell carcinomas (25% of squamous cell carcinomas and about 50% of salivary gland carcinomas less than 2 cm in diameter). There was no difference between highly and poorly differentiated types

of squamous cell carcinoma as regards the localization to the hard and soft palate nor as regards the size of the tumours at diagnosis.

The histological diagnosis of the squamous cell carcinomas in the present material offered no major problem. A few highly differentiated tumours exhibited slight cellular polymorphism but invasive growth, and recurrences revealed their malignant character. The possibility of muco-epidermoid carcinoma was considered in a few cases, but the absence of true goblet cells and negative mucine staining reaction excluded this diagnosis.

Poorly differentiated carcinomas are in general considered more radiosensitive than highly differentiated types. An attempt was therefore made to distinguish between highly and poorly differentiated carcinomas. The concept of "differentiation" commonly practised by pathologists, must, however, be said to be rather obscure and subjective. In the present material the tendency to form well defined cords and strands of epithelial cells, and to more or less obvious keratin formation, was the main basis for denoting a tumour as highly differentiated. Tumours with diffuse growth and tumours growing in cords without keratinization were registered as poorly differentiated tumours. We are aware, however, that the estimation of the degree of differentiation on these grounds is approximate, mostly because of a lack of a reproducible definition of the term "differentiation" on morphological grounds. An observation made in the survey of the material was that a number of squamous cell carcinomas appeared to be highly differentiated at the surface, whereas in the deeper invasive portions the tumour structure was rather poorly differentiated (e.g. Fig. 6). It was also observed that in some cases there was a discrepancy between the general pattern of the tumour in the sense of regular structure and formation of cords, on the one hand, and the degree of cellular polymorphism on the other. As is demonstrated in Figs. 3 and 6, a seemingly well differentiated tumour may be rather polymorphous and also show a tendency to cellular dissociation in the invasive portions. Tumours registered as poorly differentiated carcinomas in the present material all exhibited considerable cellular polymorphism, as is evident from Figs. 4 and 5.

A majority of the 123 cases with squamous cell carcinoma of the palate were treated with a combination of irradiation and surgery. Because of the long period covered by the material an evaluation of different forms of therapeutic measures is, however, of limited value.

The comparison of the incidence of recurrences, metastases and survival rates between *highly* and *poorly differentiated* carcinomas has shown fairly small differences. This may to some extent be due to the lack of clear-cut definitions for distinguishing tumours of the two types. A fairly large number of borderline, overlapping cases may thus influence the results.

Local recurrences after removal of squamous cell carcinomas were less frequent (16 out of 123 cases) than in cases of salivary gland carcinomas of the adenoid cystic type (12 out of 32 cases, Eneroth et al., 1968). Metastases, both to regional lymph nodes and distant, were, on the other hand, more frequent in cases with squamous cell carcinomas than in those with salivary gland carcinomas (Eneroth et al., 1968, 1970).

The survival rates in the present series are calculated on all the 112 patients who could be followed-up for a minimum of 5 years, thus including a number of cases in which the therapy may be considered palliative (biopsy and irradiation only). Since the number of cases receiving adequate therapy is uncertain in most materials reported (Martin, 1942, Kohn, 1961, MacComb et al. 1967), a comparison between therapeutic results is of minor interest.

The squamous cell carcinomas of the palate in this investigation have been found to be considerably more malignant types of tumours than salivary gland carcinomas. The overall 5 year determinate survival rate of the squamous cell carcinomas (41%) was sig-

nificantly lower than for salivary gland carcinomas (77%) (Hjertman & Eneroth, 1970). The difference in determinate survival rates after 20 years of observation is even more striking, 13% and 50% respectively.

In conclusion, squamous cell carcinomas of the palate have proved to be highly malignant tumours with a high frequency of metastases and a fairly low survival rate. It is demonstrated that a rapidly developing, ulcerated tumour with a diameter of more than 2 cm in the soft palate is more likely to be a squamous cell carcinoma than a salivary gland carcinoma, and this differential diagnosis is important as an early correct diagnosis may influence the choice of therapy. Improved and better defined histological criteria for morphological grading of the squamous cell carcinomas is also desirable. An investigation in this respect will be published shortly.

ZUSAMMENFASSUNG

An 383 Primärtumoren im Gaumen wurde eine histologische Untersuchung durchgeführt. Von diesen Fällen waren 219 maligne Tumoren, von denen 123 Plattenepithelkarzinome (56%) und 75 maligne Speicheldrüsentumoren (34%) waren. Die Plattenepithelkarzinome wurden entweder als hoch differenziert (83 Fälle) oder als niedrig differenziert (40 Fälle) klassifiziert; die morphologische Basis für die Unterscheidung der beiden Typen wird eingehender erörtert. Die Untersuchung zeigte, dass die niedrig differenzierten Karzinome öfter ulzeriert waren, häufiger Metastasen zeigten und einen höheren Mortalitätsgrad als die hoch differenzierten Karzinome aufwiesen. Beim Vergleich zwischen den verschiedenen klinischen Merkmalen der beiden dominierenden Gruppen maligner Tumoren des Gaumens — Plattenepithelkarzinome und maligne Speicheldrüsentumoren — zeigte es sich, dass Plattenepithelkarzinome öfter am weichen Gaumen vorkommen und im allgemeinen grösser und häufiger ulzeriert sind als maligne Speicheldrüsentumoren. Plattenepithelkarzinome weisen auch eine kürzere Symptomperiode vor der Therapie auf. Trotzdem ist jedoch die Prognose für sie weniger günstig als für maligne Speicheldrüsentumoren.

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Acta Otolaryng 73 428–432, 1972

CONCENTRATION OF AMPICILLIN IN ANTRAL MUCOSA FOLLOWING ADMINISTRATION OF AMPICILLIN SODIUM AND PIVAMPICILLIN

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(Received July 12, 1971)

Abstract Antibiotics are commonly used systemically in the treatment of sinusitis. However controlled investigations have shown that there is only slight or doubtful effect. We have therefore in connection with a clinical trial of a new antibiotic (pivampicillin, Pondocillin®) determined the tissue levels of ampicillin in the mucosa of the sinus. It has been shown that the concentration of ampicillin in nasal polyps following a single dose of pivampicillin 356 mg given orally (mean. 2.11 μg/g) is of the same magnitude as that found in nasal polyps (mean: 2.28 μg/g) and mucosa from the sinus (mean. 1.67 μg/g) after a chemically equivalent single dose of ampicillin sodium 250 mg given intramuscularly It is therefore probable that the same tissue concentration can be obtained in the mucosa of the sinus following a similar dose of [illegible]llin. Some effect of both ampicillin sodium [illegible] pivampicillin can thus be expected in the treat[illegible]ment of sinusitis.

Antibiotics are commonly used systemically in the treatment of sinusitis. A doubtful effect has been demonstrated in some investigations using various antibiotics (Kortekangas, 1963 1965) and doxycycline (Jeppesen & Schaldemose, 1970). A slight effect was seen with penicillin V tetracycline and erythromycin (Reynolds et al., 1964) On the other hand, Axelsson et al. (1970) were able to demonstrate an effect of penicillin V Some investigators were able to find a better effect from broad spectrum antibiotics than from penicillin (oxytetracycline (Lumio 1956) and lincomycin (Axelsson et al., 1970)) Others have been unable to confirm the better effect of tetracycline or erythromycin (Reynolds et al., 1964)

Concentration of antibiotics in secretion and mucosa of the sinus

In the last few years a number of investigations have been carried out on the concentration of antibiotics in the secretion and mucosa of the sinus. An apparent therapeutic concentration of penicillin V occurs in the secretion from the sinus in 60% of the cases (Gullers et al., 1969) while a higher percentage is obtained if doxycycline is used instead (Lundberg et al., 1969) Lundberg et al. (1969) were only able to demonstrate 0–4 IU/g in the mucosa of the sinus after the administration of 1.2 mill IU twice daily of benzylpenicillin procain. The specimens used for the determination were removed within 2 to 6 hours of the administration. Okuda (1968) was able to demonstrate using fluorescence microscopy tetracycline in the sinus mucosa following systemic and topical application of the compound.

PRESENT INVESTIGATION

As far as is known to the authors no investigations have been carried out of the concentration of ampicillin in the mucosa of the maxillary sinus. We considered it of interest, in connection with a clinical trial of a new antibiotic belonging to the ampicillin group (pivampicillin) used for the treatment of sinusitis, to determine the concentration of ampicillin

and pivampicillin in the mucosa of the maxillary sinus.

Pharmacology

rvampicillin, the pivaloyloxymethylester of is used as the hydrochloride salt ondocillin®) It is rapidly and effectively ab- from the stomach and upper small Ampicillin is formed in the mucosa y hydrolysis. The peak serum level of ampicil- n is therefore 2 to 3 times as high as that ined after oral administration of ampicil- and as high as that obtained after the scular administration of a chemically valent dose of ampicillin sodium (Foltz et l 1970; Jordan et al., 1970; Daehne et al., 1970).

From this it can be expected that the tissue levels in the sinus mucosa will be similar after administration of ampicillin hum and the oral administration of piv mpicillin provided the dosage is chemically valent.

MATERIAL

As resection of the maxillary sinus is carried in our department under general anesthesia and the patient therefore is fasting, the rial could not be conducted using peroral rvampicillin as was desired. Ampicillin sodium was therefore given as an intramuscular injec ion. A reference group was thus necessary 1 patients undergoing removal of nasal ps under local anesthesia were chosen. lf of these patients were treated with ampicillin sodium intramuscularly and the other alf given equivalent doses of pivampicillin.

Group 1 consisted of 3 patients on whom ection of the maxillary sinus was performed ing to chronic sinusitis unilateral resection performed on 2 and bilateral on the re- ning patient. All three were given a single of ampicillin sodium (Doktacillin®) 250 intramuscularly

Group 2 consisted of 5 patients subjected to val of nasal polyps. They were given a single dose of ampicillin sodium (Doktacillin®) 250 mg intramuscularly

Group 3 consisted of 5 patients in whom nasal polyps were to be removed and who received one peroral dose of pivampicillin (Pondocillin®)[1] 356 mg prior to the operation.

METHOD

The operation was planned so that the first tissue sample could be removed approximately $1\,{}^1/_2$ hours after the administration of ampicillin sodium, as peak tissue levels are assumed to be reached ${}^1/_2$ hour after the peak serum level which occurs 1 hour after the administration. In those cases in which pivampicillin was given the first tissue samples were removed 2 hours after the administration as it was considered necessary to allow an extra half hour for the gelatin capsule to dissolve and the pivampicillin to be absorbed. Blood samples were taken at the same time as the tissue samples in order that corresponding serum levels could be obtained. Pethidine was the only premedication used.

Transport of the samples and analysis

When a sufficiently large piece of tissue (0.25 to 1.0 g) had been removed, the blood was cleaned away using filter paper and the sample placed in a dry glass container similar to those used for the blood samples. Transport to the laboratory was by airfreight in cooled thermocontainers. The analysis could thus be carried out within 5 hours.

The ampicillin level was determined by means of the agar diffusion method, using Sarcina lutea ATCC 9341 as the test organism and ampicillin trihydrate (calculated as water free ampicillin) as the standard. The substrate was N.I.H. agar (Difco). A phosphate buffer (pH 6.8) was used for dilution. The samples were comminuted in a mortar by grinding

[1] Pondocillin® was developed and the bacteriological and statistical analyses were carried out by Leo Pharmaceutical Products, Copenhagen, Denmark.

Table I. *Group I Tissue level of ampicillin in mucosa of the maxillary sinus following intramuscular administration of ampicillin sodium (Doktacillin®), 250 mg*

Patient no.	Time between administration and removal of sample (minutes)	Serum level (C_S) (µg/ml)	Tissue level (C_T) (µg/g)
1	92		2.6
	94	4.5	
2	88		2.1
	90	2.0	
	119		1.2
	120	1 7	
3	90		1.2
	92	2.9	

with sterile sand during the addition of the buffer

RESULTS

The levels in maxillary mucosa and nasal polyps together with the corresponding serum levels are shown in Tables I to III.

Table II. *Group II Tissue level of ampicillin in nasal polyps following intramuscular administration of ampicillin sodium (Doktacillin®), 250 mg*

Patient no.	Time between administration and removal of sample (minutes)	Serum level (C_S) (µg/ml)	Tissue level (C_T) (µg/g)
1	88		2.5
	98	2.1	
2	87		2.3
	88	6.2	
	109		2.2
	110	4.2	
3	95		1.8
	97	3 4	
	126		1.5
	133	2.3	
4	86		2.5
	88	3 9	
	105		1.8
	108	3.0	
5	85		3.3
	86	4.3	
	100		3.2
	102	3.6	

Table III. *Group III Tissue level of ampicillin in nasal polyps following oral administration of pi[...] ampicillin (Pondocillin®), 356 mg*

Patient no	Time between administration and removal of sample (minutes)	Serum level (C_S) (µg/ml)	Tissue level (C_T) (µg/g)
1	115		4.5
	120	5.8	
	145		3.2
	153	5.2	
2	125		2.3
	130	2.5	
	140		1.1
	145	2.5	
3	122		1.6
	132	1.9	
	149		1.0
	150	1.0	
4	130		1.4
	132	1.8	
5	120		4.8
	124	5 4	

It has been shown using the median test that C_S (the serum level) in group 3 does no[t] differ significantly from that of a norma[l] material (Daehne et al., 1970) nor does C_T (the tissue level) deviate in this group from C_T in groups 1 and 2. The mean of C_T and C_T/C_S for the various groups are shown in Table IV.

Table IV *Mean tissue levels and mean C_T/C_S adjusted for the differences in time of sampling in the 3 groups (within 95% confidence limits)*

	Mean tissue level (µg/g)	Mean C_T/C_S
Group 1		
Maxillary mucosa ampicillin sodium 250 mg i.m.	1.67 (1 12–2.49)	0.64 (0.43–0.95)
Group 2		
Nasal polyps ampicillin sodium 250 mg i.m.	2.28 (1.91–2.71)	0.63 (0.51–0.77)
Group 3		
Nasal polyps pivampicillin 356 mg peroral	2.11 (1.37–3.25)	0.72 (0.59–0.88)

(within 95% confidence limits). The values have been adjusted for the difference in time between the removal of the tissue and the withdrawal of the blood samples.

DISCUSSION AND CONCLUSION

There is no significant difference between the mean tissue level in nasal polyps removed after the intramuscular administration of ampicillin 250 mg and pivampicillin 356 mg perorally or that of the sinus mucosa following intramuscular administration of ampicillin sodium 250 mg. It is extremely probable that the same tissue level would be obtained in sinus mucosa following the peroral administration of 356 mg of pivampicillin. The tissue level is 63 to 72% of the serum level with the dosage used and with the sampling time chosen.

If these values are compared with the minimum inhibitory concentrations for many strains of pathogenetic bacteria normally found in cases of sinusitis (Rolinson & Stevens, 1961 Sutherland & Rolinson, 1964 Williamson 1968) it can be seen, that the tissue concentration of ampicillin obtained during this trial is higher than the minimum inhibitory concentration for Pneumococci, Haemophilus influenzae Streptococci, many sensitive strains of Staphylococci, E. coli and Proteus mirabilis. It is, however lower for other species of Proteus, Pseudomonas pyocyanea, Klebsiella and Aerobacter aerogenes. Thus some effect may be expected from the treatment of sinusitis caused by the above-mentioned sensitive bacteria, provided of course that minimum inhibitory concentrations can be used in this respect.

ZUSAMMENFASSUNG

Die systemische Verwendung der Antibiotika ist bei der Behandlung von Sinusitis weit verbreitet. Kontrollierte Untersuchungen zeigen doch geringe oder zweifelhafte Wirkung. In Verbindung mit einer klinischen Durchprüfung eines neuen Antibiotikums (Pivampicillin, Pondocillin®) haben wir die Gewebekonzentrationen von Ampicillin in den Kieferhöhlenschleimhäuten bestimmt. Es ist dadurch erwiesen, dass die Konzentration von Ampicillin, die in den Nasenpolypen nach einem einzelnen oralen Eingeben von Pivampicillin 356 mg (durchschnittlich 2,11 μg/g) genau die Gewebekonzentration in Nasenpolypen (durchschnittlich 2,28 μg/g) und Kieferhöhlenschleimhäuten (durchschnittlich 1,67 μg/g) nach intramuskulärem Eingeben einer äquivalenten, einzelnen Dosis von Natriumampicillin 250 mg entspricht. Es ist deshalb überwiegend wahrscheinlich, dass die entsprechende Gewebekonzentration in den Kieferhöhlenschleimhäuten nach Eingeben von Pivampicillin gefunden werden kann. Eine Wirkung von sowohl Natriumampicillin wie von Pivampicillin ist folglich bei der Behandlung von Sinusitis zu erwarten.

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Acta Otolaryng 73 433–442, 1972

STUDIES ON THE STRUCTURE AND FUNCTION OF THE ANTERIOR SECTION OF THE NOSE BY MEANS OF LUMINAL IMPRESSIONS

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Abstract. The method introduced by Legler to obtain impressions of the anterior section of the nose has proved to be extremely valuable as far as the obtaining of information is concerned. The form and structure of the vestibulum and the anterior section of the cavum can be described more exactly than previously From this emerged a new boundary between the vestibulum and the cavum, the anatomical ostium internum. The anterior section of the nose has three important transverse areas: the external nostril, the anatomical internal nostril and the isthmus nasi The anatomical internal nostril determines in particular the direction of the air-flow through the cavum. The main function of the isthmus nasi is the formation of resistance. The main resistance-regulating effect is not found in what Mink defined as the nasal valve. Excursions of the soft parts situated below the cartilago triangularis in the incisura nasalis of the apertura piriformis are considerably more important. The particular form of the vestibulum cannot be directly compared to a funnel. Correspondingly in investigations carried out on a cadaver's nose using a rhinomanometer it could not be shown that the vestibulum had a resistance-reducing effect.

The anterior sections of the nose, including the vestibulum nasi and the anterior part of the cavum nasi, cause more than half of the total resistance in nasal respiration in the healthy individual.

Surprisingly little is known about the macroscopic anatomical structure of this area, which plays such an important part in the physiology and physiopathology of respiration. Present conceptions, including the idea of the nasal valve, need to be revised and, in some cases, corrected. The aim of this paper is to contribute to this revision and correction. It is based on an analysis of hundreds of impressions of the anterior section of the nose. These impressions were taken from living white subjects, but racial characteristics such as were investigated by Bode are not dealt with here. Due to the elasticity of the "Lastic 55" used, it was possible for the first time to take impressions from living subjects.

Impression technique

A small amount of cotton wool was placed loosely in the nose behind the limen nasi. Approximately 5 ccm of the moulding substance was then sprayed into the vestibulum nasi. This set after 3–5 min and the impression was removed carefully See Legler (1967) for further details. These impressions made it possible to give a more exact description of the form of the vestibulum and of the anterior section of the cavum, and of the boundary between these two In addition, the transverse surfaces of the exterior nostril, the anatomical ostium internum and the isthmus nasi, which are important in the respiratory air flow can be defined and measured.

Vestibulum nasi

It was noticeable in all the impressions that there was a small "snout-like" formation—the vestibulum nasi—attached to the cavum like a "filler pipe" This can be clearly distinguished from the cavum by its form and axial direction and it is obviously a separate



Fig 1 Luminal impression of the anterior section of the nose from the side. Groove *a–b* plica vestibuli, *a–b–c* limen nasi – lateral boundary line between the vestibulum and the cavum nasi. (*d*) Impression of the crus laterale of the alar cartilage. (*e*) external nostril.

anatomical unit (Figs. 1 2, 3 4). The question of where the vestibulum ends and the cavum begins has long been a topic of discussion. This can be seen quite clearly on the impressions. The vestibulum begins at the external nostril and ends at the cavum in a transverse area, which up to now has not been described. This is distinguished by macroscopic struc



Fig 2. Luminal impression of the anterior section of the nose from the septum. (*a*) sharp ridge which forms the barrier between the vestibulum and the cavum at the septum.

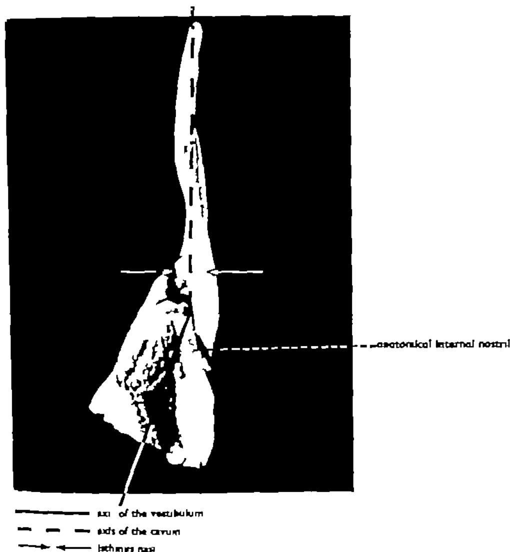


Fig. 1. Luminal impression of the anterior section of the nose from the front. Below the anatomical internal nostril in black is the vestibulum, above this is the cavum. The axes of the cavum and the vestibulum form an acute angle.

tures which are invariably demonstrable and which form a boundary complete in itself.

On the lateral side of the impression the boundary is formed by the curved limen nasi (Fig. 1). This consists of the plica vestibuli the upper part of which is almost horizontal, and a somewhat less well-defined continuation which branches downwards. This opens into a groove which crosses the base of the nose (Fig. 2). The boundary line on the septal side of the impression is formed by a sharp ridge which runs upwards at the front and at the dorsum nasi meets the origin of the plica vestibuli (Fig. 2).

From the histological point of view the boundary on both the lateral and septal sides lies in infancy exactly in the transitional area between the squamous epithelium and the ciliated cylindrical epithelium. Later in life the boundary in the septal region between the squamous epithelium and the respiratory epithelium is gradually slightly displaced in the

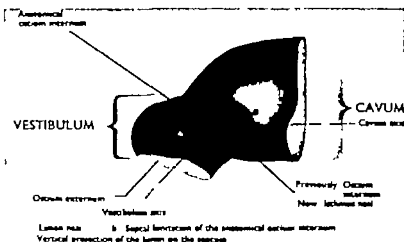


Fig. 4 Diagram of the anterior section of the nose based on a luminal impression.

direction of the cylindrical epithelium due to metaplasia.

It was also shown that this boundary had functional characteristics (see below) and this confirmed the newly defined demarcation line between the vestibulum and the cavum from the macroscopic, histological and functional points of view

It must therefore be established that con ry to previous views, the vestibulum does end at what Zuckerkandl (1882) called e ostium internum. This is defined as a slot like space between the limen nasi and the septum (Fig. 3) and its area is therefore per pendicular to the axis of the cavum and is therefore part of the cavum and independent of the vestibulum axis (Fig. 4) In addition, Zuckerkandl's definition of the ostium in ternum was not completely satisfactory be cause no boundary structure for the ostium could be found on the septum side.

Unfortunately terminological difficulties are involved in this new definition of the boundary between the vestibulum and the cavum. The structures which were previously defined as the boundary surround an area which is almost flat and which forms an orifice, predominantly lateral, in the cavum. Logically this orifice should therefore be de scribed as the internal nostril. In order to avoid confusion and to render the terminology as unambiguous as possible, Legler (1968) suggests that the term anatomical ostium internum should be adopted. It would be more practical if the term ostium internum employed previously were not used without the name of its inventor Zuckerkandl (1882). A better solution however would be to refer to this as the isthmus nasi since it forms the narrowest part of the anterior section of the nose (Figs. 3 4)

The form of the vestibulum is of decisive importance for its functional characteristics. With regard to this, several details of form which are particularly evident in the impressions are described below

1 The vestibulum is a flat, oval structure, as can be seen from the transverse areas of the external and internal nostrils forming the boundaries of the vestibulum. The anatomical internal nostril is more rounded than the external nostril.

2. The anterior wall of the vestibulum is hollowed by the cavities of the tip of the nose.

3 There is a constriction of the lumen of the vestibulum caused by a wide groove which runs along the lower edge of the crus laterale of the alar cartilage, downwards at the front and upwards at the back, and opens into the limen nasi (Fig. 1).

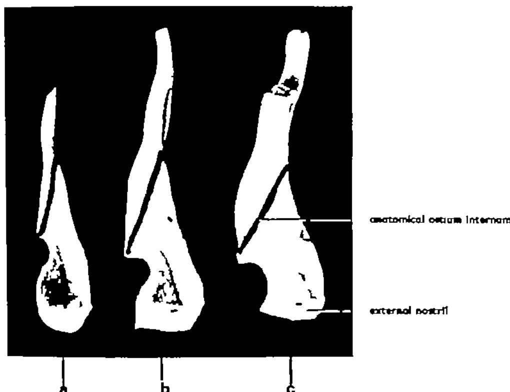


Fig. 5 Luminal impression of the anterior nose. Frontal section in the direction of the vestibulum axis (*a*) through the anterior (*b*) through the central, and (*c*) through the posterior third of the vestibulum. In (*b*) and (*c*) there is no funnel-like tapering from the external nostril to the anatomical internal nostril.

4. On the frontal section parallel to the vestibulum axis through the anterior central and posterior thirds of the vestibulum it can be seen that there is a constriction from the external nostril to the anatomical ostium internum only in the anterior third. The cut surface through the central third showed this type of constriction to a limited extent in only one-third of all the cases investigated. In the posterior third, on the other hand, an enlargement could be seen in most cases (Fig. 5). Thus, since the form of the vestibulum differs in several respects from that of a funnel, these results raise some doubts as to whether the vestibulum can be assumed to have a funnel action, i.e. whether it does lower the resistance.

5 The vestibulum is at an oblique angle to the cavum. The angle formed by the axes of the vestibulum and the cavum can only be calculated approximately because the particular construction of the vestibulum results in a bent central axis. If the centre of the external nostril is joined to the centre of the anatomical ostium internum by a straight line, this axis forms an average angle of 50 –80 with the axis of the cavum, when viewed from the side (Fig. 4). When viewed from the front, it forms an angle of 15 –30 with the septum (Fig. 3)

Structure of the anterior section of the cavum

The topography form and size of the anatomical ostium internum and of the isthmus nasi can only be described in detail and understood properly by imagining the structure the anterior section of the cavum. Mink (1 compared the cavum to a bor...

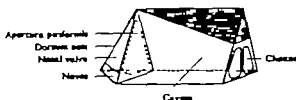


Fig 6 Diagram of the nasal cavity according to Mink. Pyramid – vestibulum, triangular prism – cavum.

sided prism. Its anterior surface is formed by the apertura piriformis. The vestibulum is situated in front of this main cavity and can be imagined as a three-sided prism (Fig. 6) This model gives a misleading picture. As the luminal impressions show it is not merely the upper edges of the cavum which run together to form a point but also the anterior edges, and the base is rounded. The result is an almost stream-lined structure which can be compared to the tip of an aircraft wing (Fig. 4)

The anatomical ostium internum

If the vestibulum is separated from the cavum, the typical position of the anatomical ostium internum on the cavum can be seen i.e. in front below and lateral. The surface of the is almost flat and, in comparison with xternal nostril, is set somewhat steeply (Fig. 3) It has almost the same position in relation to the surface of the septum as the tympanic membrane to the sagittal plane of the body From the front (Fig. 3) and also from below (Fig. 7) it forms an acute angle with the septum.

The form and size of the anatomical ostium internum is affected on the one hand by the particular structure of the vestibulum, as described above, and on the other by the fact that the vestibulum is attached obliquely to the cavum. Measurements carried out by Schmitt & Fuchs (1968) showed that the area of the anatomical ostium internum is on average 1 4–1 6 times larger than that of the external nostril. It also has a larger curve.

The hydraulic diameter important for the flow is 1.2 times larger than that of the external nostril.

Isthmus nasi

The isthmus nasi, the third important transverse surface apart from the external nostril and the anatomical internal nostril, can be seen in the impressions by making an incision along the limen nasi perpendicular to the septum, according to Zuckerkandl's definition. This type of incision will show immediately that the ostium internum as defined by Zuckerkandl is a transverse area of the cavum and therefore cannot represent the boundary of the vestibulum (Fig. 4).

Since the limen is in the shape of an arch,



Fig 7 Luminal impression of the anterior section of the nose from below (*a*) anatomical ostium internum, (*s*) septum axis, (*o*) long axis of the anatomical ostium internum. These two axes meet to form an acute angle.

the surface of the isthmus nasi is curved (Figs. 4 12). Thus the question arises as to whether the value of the whole surface area can be used in the formulae for the calculation of the nasal resistance, or only the value of its frontal area.[1] According to Zuckerkandl the ostium internum is the narrowest passage through which the air passes in the anterior section of the cavum.

The surfaces of the external nostril, of the anatomical internal nostril and the isthmus nasi show approximately the following proportional ratio to each other 1 1 4 0 7 i.e. the isthmus nasi is half the size of the anatomical ostium internum and approximately two-thirds the size of the external nostril.

Function

1 As described above, the anatomical ostium internum is the inlet opening into the cavum. It can be seen from diag. 3 that the air can flow into the cavum through the anatomical ostium internum from the front, from below and from the sides. The largest inflow occurs laterally· inflow from below is less and inflow from the front is the smallest. This can be seen from the diagram of each of the frontal areas (Fig. 8). Since the anatomical ostium internum was not known, until now the lateral inlet has not been considered.

Thus the frontal inlet (Mink, 1920), the inflow at the base (Proetz, 1953 v Dishoeck, 1936) and the inlet at the front and below (Tonndorf) as represented in previous flow-models do not correspond to the actual nose. Thus, for example, on Mink's box-model, the anterior corner should be cut off obliquely (Fig. 9 *a*)

The site and setting of an inlet opening in a hollow structure is, to a great extent, the determining factor in the flow direction of the air intake. This can also be shown in the case

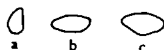


Fig 8 Difference in the size of the frontal area of the anatomical ostium internum caused by the different air flow directions. (*a*) from the front, (*b*) from below (*c*) from the side.

of the cavum, as demonstrated in the following test

Smoke was blown through a transparent model of the nose, without the conchae but with a true-to-life anterior cavum section and vestibulum (based on a luminal impression taken from a live subject). The experiment was repeated after the vestibulum had been separated at the anatomical ostium internum. It was seen that the flow direction of the air in the cavum was determined by the site and setting of the anatomical ostium internum (Fig. 10 *a*). The flow direction was not affected by the external nostril being directed downwards (Fig. 10 *b*)

2 As described above, the vestibulum is a flat, oval formation which has special features

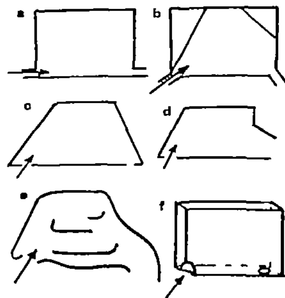


Fig 9 a) Box-model according to Mink. b) according to Tonndorf c) according to Sercer d) Proetz, e) according to van Dishoeck, f) Mink's model with the correct inlet opening.

[1] The frontal area is obtained by projecting the curved surface onto a plane. The projection direction corresponds to the flow direction; the projection plane is perpendicular to the flow direction.



Fig 10 (*a*) Model of the cavum without vestibulum. Smoke has an oblique inflow direction through the anatomical ostium internum. (*b*) Model of the cavum with true-to-life vestibulum. The inflow direction of the smoke is not affected by the external nostril.

of form. A tapering of the lumen from the external nostril to the anatomical internal nostril could not be observed over the whole length of the transverse section. The comparison with a funnel is therefore oversimplified and incorrect. Tests were carried out on a cadaver's nose with a rhinomanometer to determine whether as suggested by v Dishoeck (1936) the vestibulum has a resistance-lowering effect. As can be seen from Fig. 11 no increase in the resistance coefficient could be demonstrated after the vestibulum had been removed at the anatomical ostium internum.

3 The hydraulic diameters of the external nostril, the anatomical internal nostril and the

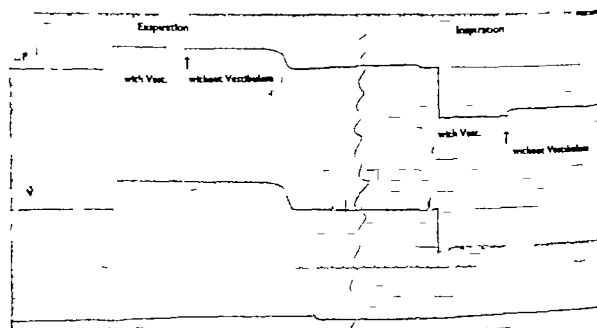


Fig. 11 Rhinomanogram of a cadaver's nose through which air is passed. During both inspiration and expiration the respiratory resistance is lowered after removal of the vestibulum.



Fig. 12 Left: view of the curved isthmus nasi in a luminal impression. Right, surface of the isthmus, shown as flat when rolled off on paper Smaller area of flow in the region of the nasal valve.

isthmus nasi show an average proportional ratio of 1.2 1 4 0.8. Comparisons with a larger series of rhinomanometric investigations would have to be carried out in order to show whether deviations from these ratios allow any definite information on nasal respiration obstructions occurring in the anterior section of the nose.

On the other hand, it can be seen from the above proportions that the anatomical internal nostril plays the least important part in the development of resistance. It is mainly an inlet opening in the cavum and its function is to determine the flow direction.

The resistance occurs in particular in the isthmus nasi. The regulation of the resistance is carried out mainly by alterations in the size of the isthmus nasi and the external nostril. This was shown by a comparison of the sizes in the three transverse surfaces in the normal state and also after active enlargement of the anterior section of the nose During active enlargement, it was the areas and the hydraulic diameters of the external nostril and the isthmus nasi which increased in particular On the other hand there was a much smaller increase in the large, circular anatomical ostium internum (Riedel, 1968)

4 Mink (1920) maintained that in order to regulate the respiratory resistance the isthmus nasi was altered by the action of the muscles of the external nose in particular in the area of the nasal valve (= cartilago triangularis, in particular its lower edge) Up to the present this has not been contradicted but our investigations have made it necessary to review the term nasal valve.

If the area of the isthmus nasi is cut out of an impression, the area of Mink's nasal valve can easily be illustrated on this. It can be seen that it only forms the smaller portion of the total transverse surface (Fig. 12) It therefore seemed doubtful whether this would contain the main regulatory mechanism for the nasal resistance In fact, comparative investigations with a rhinomanometer showed that a particular amount of pressure on the nasal valve caused a smaller increase in the resistance than the same amount of pressure on the soft parts situated below this. The functional char

acteristics of the latter have not yet been taken into consideration. The theory that the nasal valve is the most important factor in the regulating of the nasal resistance in the anterior region of the nose can be discounted on the basis of the results obtained from these measurements.

It is therefore necessary to provide a new definition of the nasal valve. It cannot be defined merely as the lower edge of the cartilago lateralis, but rather the whole of the mobile lateral wall should be considered as the functional unit in the regulation of the nasal resistance.

Due to its special structure and in particular due to the presence of the soft parts mentioned above the limen nasi is completely mobile along its whole length and thus rapid transverse alterations are possible in the isthmus nasi, in particular along the lower part.

ZUSAMMENFASSUNG

Die von Legler eingeführte Abdruckmethode des vorderen Nasenabschnittes hat sich als sehr aussagekräftig erwiesen. Form und Bau des Vestibulum und des vorderen Cavumabschnittes konnten genauer als bisher beschrieben werden. Daraus ergab sich eine eine Abgrenzung des Vestibulum vom Cavum durch sogenannte anatomische Ostium internum. Es ieren drei wichtige Querschnittsflächen des vorderen Nasenabschnittes: Das äußere Nasenloch, das anatomisch innere Nasenloch und der Isthmus nasi. Das anatomisch innere Nasenloch bestimmt vor allem die Einströmrichtung der Luft in das Cavum. Widerstandsbildend wirkt überwiegend der Isthmus nasi. Dem Bereich der Mink'schen Nasenklappe ist nicht der stärkste widerstandsregulierende Effekt zuzusprechen. Excursionen der caudal vom Dreieckknorpel in der Incisura nasalis der Apertura piriformis liegenden Weichteilfläche kommt wesentlich größere Bedeutung zu. — Die Formbesonderheiten des Vestibulum erlauben keinen direkten Vergleich mit einem Trichter Entsprechend konnten wir einen widerstandsenkenden Effekt des Vestibulum rhinomanometrisch an der Leichennase nicht nachweisen.

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Acta Otolaryng 73 443–452, 1972

ABNORMAL INTRAEPITHELIAL GLANDS IN THE HUMAN TRACHEA

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(Received July 14 1971)

Abstract. Among [illegible]5 tracheae studied systematically for intraepithelial glands we found in one patient —a heavy smoker and former miner—more than 5000 intraepithelial mucous glands of a type which does not appear to have been reported previously. The glands were lying in clusters of 20–80, as solitary glands, in rows, or in festoons. The density of the glands within the clusters ranged from 63 to [illegible] glands/mm². The number and density of the glands in the different parts of the trachea were greatly varied, the number being smallest in the membranous wall, largest in the lowest part of the cartilaginous wall. The architecture of the individual glands is described. It is composed of 30–50 mucous cells which are grouped especially at the bottom of a duct-shaped lumen which debouches on the surface of the trachea. The glands must be abnormal. It is endeavoured to explain the mechanism of their origin which is similar to the development of the normal submucous glands in the trachea. The formation of the intraepithelial glands may be a metaplasia of the epithelium due to a chronic irritant, possibly heavy smoking or long-lasting exposure to dust. The secretory capacity of the intraepithelial glands was calculated. It was small, amounting to 1/200–1/300 of the total secretory capacity of the goblet cells which was also increased in our patient.

The mucus-producing elements of the trachea and lower respiratory tract are mucous glands in the submucosa and goblet cells in the epithelium. The glands are of the tubulo-acinous type, consisting of the excretory duct running through the mucosa and dividing in the submucosa into two lateral ducts into which several tubules, lined with PAS-positive mucous cells, debouch. At the end of each tubule there are acini lined with mucous and serous cells. The gland mass in the cartilaginous wall is localized exclusively to the submucosa (Tos, 1969 1970 *a*) the majority in the intercartilaginous spaces where the glands are arranged in 2–6 rows. In the membranous wall the glands are arranged in several layers. Quantitative studies of the tracheal glands from 20 adults by the whole mount method have shown an average of 818 glands in the membranous and 3 123 in the cartilaginous wall, corresponding to a density of 0 7 gland/mm² in the membranous and 0 88/mm² in the cartilaginous wall. The glandular orifices were regularly distributed in the mucous membrane (Tos, 1970 *b* 1971) In chronic bronchitis hypertrophy of the mucous glands has been found (Reid, 1960· Thurlbeck et al., 1963 Restrepo & Heard, 1963).

The goblet cells are irregularly distributed in the epithelium. In an autopsy material of normal persons we (Ellefsen & Tos, 1971 *a*) found a goblet-cell density of 128 ± 28 per counting field of 0 01768 mm² ranging from 100 to 150 cells per field, corresponding to 5 600 and 8 500 cells/mm² Under pathological conditions we found in a biopsy material (Ellefsen & Tos, 1971 *b*) an increased density of goblet cells, viz. in acute tracheobronchitis an average of 141 goblet cells per field, in mild chronic tracheobronchitis 145 and in typical severe chronic bronchitis 180 cells per field.

The object of this paper is to present an-

other form of mucous elements in the trachea—intraepithelial mucous glands. These glands differ materially from the normal tracheal glands in their situation, architecture size, shape, distribution and density Despite several systematic studies on the tracheal mucosa under normal and abnormal conditions (Frankenhaeuser 1879· Hess, 1936 von Hayek, 1953 Turunen, 1955 Gläser 1957 Reid, 1960) such glands have never previously been described in the human trachea. Clara (1937) mentioned intraepithelial glands or nests of goblet cells, but in his terminology this meant epithelial areas with a particularly great goblet-cell density not actual glands. Järvi (1935) found no intraepithelial glands in systematic studies of the mucous membrane of the larynx and trachea of certain mammals.

Intraepithelial glands have been found in the nasal mucosa (Zarniko, 1894 Boenninghaus, 1895 Goerke 1897 Okada, 1898 Cordes, 1900· Zarniko, 1903 Glas, 1904 Hajek, 1905 Oppikofer 1907). Originally it was believed that they occurred only under abnormal conditions, especially in a hypertrophic nasal mucosa. Boenninghaus (1895) found these glands in two cases among 20 systematically studied patients with nasal polyps—numerous glands in one case and one gland in the other Moreover this author described two cases of foetid atrophic rhinitis with intraepithelial glands. Zarniko (1903) among 22 patients with nasal polyps, found four with intraepithelial glands, situated in the inferior concha or in the stalk of the polyp. Glas (1904) found intraepithelial glands in 12 cases out of 120 with hypertrophic rhinitis. Oppikofer (1907) studying the nasal mucosa from 200 individuals, found intraepithelial glands in 164 and many patients of this group had an normal nasal mucosa. Thereafter it was believed that the intraepithelial glands were a normal component of the nasal mucosa, but this view has neither been proved nor disproved in the more recent literature (Messerklinger 1958).

Intraepithelial glands have also been described in the human Eustachian tube (Citelli, 1905), epiglottis (Patzelt, 1923) and in the sinus of the larynx (Citelli, 1905). They have been described also in certain parts of the alimentary tract of some mammals (Boenninghaus, 1895)

MATERIAL AND METHODS

The trachea to be described below was examined in the course of a consecutive study aiming at elucidating the distribution and density of goblet cells in normal persons and in patients with various respiratory diseases. The material comprised 25 patients most of whom had died of acute cardiac diseases or malignancies. The patient in whom we found the intraepithelial glands was a 65-year-old man who had died of acute coronary occlusion. In 1924 he had had pneumonia, for many years he had been working as a miner in Peru, and had been a heavy smoker 60 cigarettes daily through 40 years, but during the last year 12 cigarettes a day Autopsy revealed arteriosclerosis of the coronary arteries, cardiac infarcts, and signs of pulmonary congestion. Otherwise, the patient had not had manifest acute or chronic diseases of the respiratory tract, and no such signs had been found by physical or radiographic investigations during his last stay in hospital. Apart from the pulmonary congestion resulting from the acute heart disease autopsy showed no striking signs in the lungs or lower respiratory tract.

The procedure of the study was to fix the trachea 2–5 hours after death by formol alcohol injected into its lumen through the cricotracheal ligament. At autopsy 12–24 hours later the trachea was removed and fixed again for at least 24 hours. Thereafter it was dissected and cut along the membranous wall. In order to stain the mucosa as well as the submucosa, the trachea was microdissected, separating the mucosa with the lamina prop-

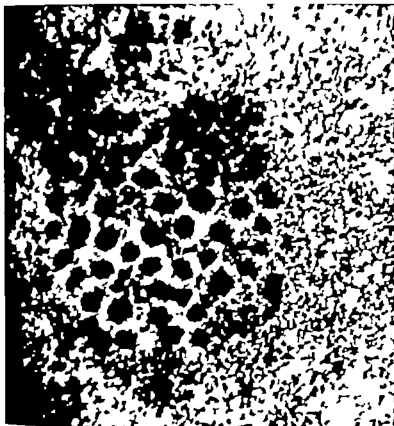


Fig. 1. A cluster with a great density of intraepithelial glands from the lowest quarter of the cartilaginous wall of the trachea. The glandular orifices are visible. Great density of goblet cells in the epithelium surrounding the cluster, less density within the cluster. Whole mount, ×100.

sa in one piece from the submucosa. The submucosa was further microdissected, separating and removing the fibrocartilaginous fascia with the cartilage rings, perichondrium, and annular ligament.

The mucosa and submucosa were stained by the PAS-alcian blue whole-mount method (Tos, 1970 *b*) and studied separately in the stereomicroscope magnified ×40 to detect abnormal glands in the epithelium and study the normal glands in the submucosa. The goblet cells were studied quantitatively according to principles described previously (Ellefsen & Tos, 1971 *a*).

After the trachea had been studied by the whole-mount method, various parts of it were cut into 5 μ thick serial sections, stained with PAS-alcian blue and haematoxylin-eosin, and the structure of the glands was studied.

RESULTS

Among the 25 tracheae studied, intraepithelial glands were found in only one. The grouping of these glands in clusters (Fig. 1) is so characteristic that they can hardly escape detection with the present whole-mount method. The description of the structure and distribution of the glands is based upon investigations of sections and whole mounts, the quantitative studies of the intraepithelial glands and the determination of the goblet-cell density on the whole mount method.

Structure and situation

All the intraepithelial glands were situated within the epithelium, reaching down to the basement membrane (Fig. 2) which was some



Fig 2 Two intraepithelial glands in a greatly thickened columnar ciliated epithelium. At the bottom of the glandular lumen the mucous cells are arranged radially and surrounded by very elongated ciliated cells. Section, ×650.

what bulgy in these sites, but the glands did not penetrate the basement membrane to the lamina propria. They were 90–100 μ in length and 40–50 μ in diameter The gland usually ran a vertical, but at times somewhat oblique course down into the epithelium (Fig. 3). In its surroundings the epithelium was usually thickened, but columnar and ciliated (Fig. 2) The gland is of a fairly simple structure debouching to the surface by a narrow round orifice whose edges are lined with cilia. The orifice leads to the lumen of the gland which reaches to its bottom where the greater part of the mucous cells are situated (Fig. 4). There are 30–50 mucous cells in each gland. The luminal part of the cell is filled with mucin, and its nucleus is displaced basally At the bottom of the gland the mucous cells are *not interspersed by any supporting or ciliated* cells, but these cells are arranged around the mucous cells, extending in an arch from the basement membrane to the glandular orifice.

The special arrangement of the mucous cells at the bottom of the duct and the very elongated ciliated and supporting cells surrounding the mucous cells afford some hint of the mechanism of origin of the glands, especially if compared with the development of the normal submucous glands which form from the 11th to the 25th menstrual week. It has been demonstrated (Tos, 1966) that the basal cells of the epithelium divide a few times, giving rise to an intraepithelial, spherical glandular primordium. On further division the primordium enlarges, it grows like a solid cylinder down through the lamina propria to the submucosa where it undergoes dichotomous division several times, giving rise to several tubular primordia. The differentiation of the *primordial cells into mucous cells* commences when the primordium is 11 days old by the formation of mucin which is secreted into the



Fig 3 Intraepithelial glands within a cluster seen from above. The orifices of the vertical and the duct-shaped lumina of the oblique glands are visualised with radially arranged mucous cells. Whole mount, 500)

intercellular spaces. Thus, there arises in the middle of the solid cylinder a mucin-filled pond which widens into a canal. The canal extends subepithelially pushes the epithelial cells apart, and debouches into the tracheal lumen.

Thus, the normal submucous glands of the trachea do not arise by invagination of the epithelium, but by differentiation of the cells of the glandular primordium and secondary canalization. In a similar way the formation of the intraepithelial glands may be explained. As a result of an unknown irritant possibly as a link in epithelial metaplasia, differentiation of the basal into mucous cells commences in some parts of the tracheal epithelium. Thereby a sphere consisting of several mucous cells is created deep down in the epithelium. These cells produce mucin which is secreted into the intercellular spaces, so that between the cells a mucin filled pond arises. By continued mucus production the pond extends spreading along ciliated and supporting cells towards the epithelial surface. The cells spread out, the pond is evacuated into the tracheal lumen and the canal and orifice of the gland have come into being. On further reproduction of the mucous cells the gland widens at the bottom of the canal and acquires its final shape. Accordingly the cells surrounding the gland get very elongated and narrow, even their nuclei elongate and adapt themselves to the glandular surface. These cells form practically a shell like lining of the gland (Fig. 2) reminiscent in [illegible] a gustatory bulb

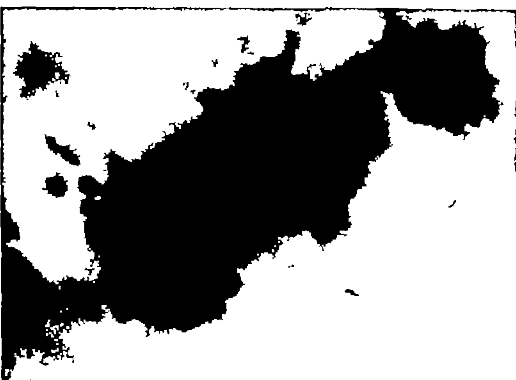


Fig 4 Intraepithelial glands from an epithelial in vagination lateral view The radial arrangement of the mucous cells around the lumen is apparent Whole mount, ×500

Distribution number and density

ithelial glands are distributed rather ir , lying either in clusters (Fig. 1) like small islets in the mucosa or as solitary glands.

By quantitative studies we found 34 such islets in the mucosa where the glands were particularly dense. In diameter these islets ranged from 0 3 to 0.7 mm corresponding to an area of 0 1 mm² and 0 4 mm² The number of glands in each islet varied from 20 to 88. Their density within each islet varied within wide limits, from 65 glands/mm² to 250 glands/mm² One islet was particularly large, having a diameter of 1 6 mm and an area of 2 mm² Within this islet there were 134 glands, density 67 glands/mm² Fig. 5 shows that in the entire membranous wall there was only one islet, in the upper quarter of the cartilaginous wall none, in its second quarter a few while the great majority were in the third quarter especially in its anterior middle part.

Quantitative studies disclosed a total of 5 218 abnormal glands. Only 1 137 were localized within the named 34 glandular islets, the remainder scattered in the tracheal mucosa, either as solitary glands, as small groups of 2–10 glands, or arranged in small rows or festoons (Fig. 6) In the membranous wall there were a total of 106 abnormal glands, in the cartilaginous wall 5 112, with fairly marked differences in number between the upper and lower quarters (Fig. 5) but the largest number in the third quarter In the cartilagmous wall there were also differences between the various sides In the middle, anterior part 2 599 in the left side 1 365 and in the right side 1 148 glands.

The density of the abnormal glands varied widely within the individual islets where the density ranged from 65 to 250 glands/mm² and between the various parts of the trachea. In the upper quarter of the cartilaginous wall the mean density of intraepithelial glands was

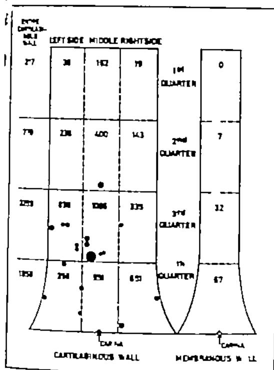


Fig. 5. Schematic presentation of the situation and distribution of the clusters (dark spots) which show a particular density of intraepithelial glands. The trachea is divided into four quarters, and the cartilaginous wall also into 12 equally large areas. The total number of intraepithelial glands in each quarter and in each area, is shown.

0.2 gland/mm² in the second quarter 0.6 gland/mm² in the third quarter 2.0 glands/mm² and in the fourth quarter 1.8 glands/mm². Thus, the density in the third quarter was ten times that in the first. In the membranous wall the mean density was minimal, 0.03 gland/mm² in the second, 0.1 gland/mm² in the third, and 0.13 gland/mm² in the fourth quarter.

In the trachea there were some invaginations of the epithelium (Fig. 7) where the epithelium lined 2 × 3 mm cavities in the tracheal wall. The invagination had a slit-shaped opening to the tracheal lumen. Its epithelium is columnar ciliated epithelium. In all the invaginations, especially at their edges, there were many intraepithelial glands.

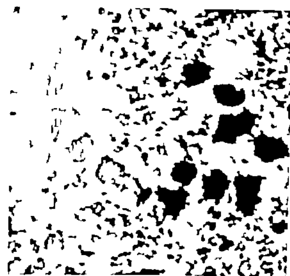


Fig. 6. Festoon-shaped arrangement of intraepithelial glands. Whole mount, × 90.

Secretory capacity of the intraepithelial glands

To establish on a quantitative basis whether all normal glands were situated in the submucosa and all the abnormal glands in the epithelium, control counting of the normal tracheal glands was carried out, of the orifices in the mucosa as well as of the glands proper in the submucosa. The number and density of the glands proved to be within the range of normal (Tos, 1966, 1970*b*, 1971), as in this trachea there were 3 353 orifices or 3 290 glands in the cartilaginous wall and 847 orifices or 867 glands in the membranous wall, corresponding to a density of 0.74 gland/mm² and 0.50 gland/mm² respectively. Thus, the presence of the intraepithelial glands had no influence upon the number of the normal submucous glands in the trachea.

The mean goblet-cell density studied in 160 fields of 0.01768 mm² distributed on 16 localities (Fig. 5), proved to be 149 ± 16.2 cells per field, corresponding to 8 4-0 cells per mm². In the entire epithelial area of the trachea 5 960 mm² there are thus about 50 [illegible] goblet cells.

The secretory capacity of the [illegible]

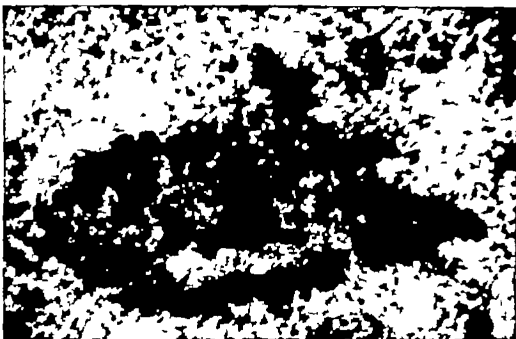


Fig 7 Epithelial invagination with a slit-shaped lumen (*arrow*) and numerous intraepithelial glands, especially along the edges of the invagination. Whole mount, × 100

glands may be compared with that of the goblet cells. In each of the intraepithelial glands there were 30–50 mucous cells. In other words, all the intraepithelial glands contain 56 000–260 000 mucous cells. Provided that the secretory capacity of the mucous cells in the glands is the same as that of the goblet cells, the abnormal glands make up only 1/200–1/300 of the total secretory capacity of the goblet cells.

DISCUSSION

There is little doubt that the intraepithelial mucous glands are abnormal. Their distribution in clusters is so irregular their density and number in the various parts of the trachea so heterogeneous that they can hardly be a normal component of the tracheal epithelium. In studies of the normal tracheal glands in 200 foetuses (Tos, 1966), 13 prematures and children (Tos, 1969 1970 *b*) and of 20 adults (Tos, 1970 *a* 1971) by the same whole mount method, no intra-epithelial glands were found. They have also not been described in previous systematic studies of sections from normal (Frankenhaeuser 1879 Turunen 1955) or abnormal tracheae (Gläser 1957 Reid, 1960). It would seem, therefore that the intraepithelial glands are not common under abnormal conditions either However only a few abnormal tracheae have been studied by the whole mount method which permits examination of the entire mucosa. To study the entire mucosa by 5 μ sections would require many thousand sections, and this might well explain why they have not been found previously In the present material attention was indeed fixed on the occurrence of intraepithelial glands, but the great majority of the 25 tracheae were normal. Therefore this material can also not form the basis of a determination of the frequency of intraepithelial glands under normal and abnormal conditions. Another biopsy material (Ellefsen & Tos, 1971 *b*) composed of 103 biopsies from 50 patients 11 of whom had mild, 11 typical severe chronic tracheobronchitis, and 8 acute changes of the tracheal mucosa, revealed no intraepithelial glands, although the

mucosa was examined by the whole mount method. However since the size of the biopsies was only a few square mm, it cannot be proved that no intraepithelial glands were present elsewhere in the trachea. To establish the frequency and cause of the intraepithelial glands a major number of abnormal tracheae have to be studied by the whole mount method.

The present patient had been a heavy smoker and besides he had been a miner. These two aetiological factors may have contributed to the formation of intraepithelial glands. The mean goblet-cell density of 149 cells per field was increased (Ellefsen & Tos, 1971 *a*, 1971 *b*), indicating an irritative condition of the mucosa. The formation of the intraepithelial glands may be due to a kind of metaplasia of the epithelium, as is indeed indicated by the great density of glands in the described clusters (Figs. 1–5). Metaplasia of respiratory epithelium into squamous epithelium is not an uncommon finding in heavy smokers (Chang, 1957), and incidentally it is well-known in the lower respiratory tract.

Messerklinger (1958) has experimentally induced intraepithelial glands in the guinea-pig trachea where they are normally not present. As early as 40 minutes after the injection of pilocarpine, which has a parasympathicomimetic effect, he found some cohering cells containing mucus in some places of the basal part of the epithelium. 20 minutes later there were fully developed intraepithelial glands, and in another 30 minutes the glands had again disappeared. In his opinion, however the intraepithelial glands were not interpretable merely as a transient increase in the secretory epithelium due to a strong acute stimulus, since with chronic stimulation they are stable structures. As a link in resuscitative measures our patient had received an intravenous drip of noradrenalin for 3 hours before death. This had also been done in several other cases of the present material, in whom no intraepithelial glands were demonstrable.

As stated above, the intraepithelial glands increase the secretory capacity of the epithelium only negligibly. The increase corresponds to 260–440 goblet cells/mm² or 5–8 cells per counting field. In this patient then a goblet cell density of 154–157 cells per field, but without intraepithelial glands, would mean the same secretory capacity as the density of 149 cells per field with intraepithelial glands. In one trachea of the material we found a mean goblet-cell density of 196 cells per field. Similarly in several biopsies from patients with chronic bronchitis we have found a goblet-cell density of about 200, maximum averaging 243 cells per field. These findings indicate that the secretory capacity of the epithelium is far from being fully utilized by the increase in goblet-cell density in the described trachea. Thus, the formation of the intraepithelial glands cannot be a simple measure to increase secretion. Its cause must be more complex, and the stimuli which result in an increase in goblet-cell density are probably not the same which cause the formation of intraepithelial glands.

ZUSAMMENFASSUNG

Über das Vorkommen von intraepithelialen Drüsen wurden 25 Luftröhren systematisch untersucht. Bei einem Patienten, der Minenarbeiter war und einen grossen Zigarettenverbrauch hatte, wurden mehr als 5 000 intraepitheliale muköse Drüsen gefunden. In der Luftröhre wurden diese Drüsen bisher noch nicht beschrieben. Sie liegen entweder haufenweise zusammen — in Ansammlungen von 20 bis 80 Drüsen — oder einzeln, oder in Reihen oder in Girlanden angeordnet. Die Dichte der Drüsen innerhalb der Haufen variiert von 65 bis zu 250 Drüsen mm². Die Zahl und die Dichte der Drüsen in den verschiedenen Abschnitten der Trachea ist ziemlich variabel. In der Pars Membranacea gibt es nur wenige, in dem distalen Abschnitt der Pars Cartilaginosa sehr viele. Der Bau der einzelnen Drüse wird beschrieben. Sie bestehen aus 30–50 mukösen Zellen, die besonders in dem Boden eines gangähnlichen Lumens, das auf der Oberfläche des Luftrohres mündet, angeordnet sind. Die Drüsen sind wahrscheinlich pathologische Gebilde. Es wurde versucht, den Entwicklungsmechanismus der Drüsen zu erklären, er gleicht der Entwicklung der normalen, seromukösen Drüsen der Luftröhre. Die Bildung der intraepithelialen Drüsen ist wahrscheinlich eine Metaplasie des Epithels infolge eines kronischen Irritamentes, vielleicht wegen des

grossen Zigarettenverbrauchs oder einer langdauernden Staubeinwirkung. Die Sekretionskapazität der intraepithelialen Drüsen wurde ausgerechnet, sie ist klein und beträgt 1/200–1/300 der gesamten Sekretionskapazität der Becherzellen, die bei diesem Patienten auch erhöht ist.

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Acta Otolaryng 73 453–454 1972

THE OTOMICROSCOPE AND MICROSURGERY
1921–1971

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"From Barber to Surgeon" was the title of a paper recently read by Olle Hultén, the former professor of surgery at Uppsala University in Sweden. He described among other things the often rugged practice of surgery *before* the 19th century Recently I read a book by the German author Jürgen Thorwald (1969) about "The century of Surgery" describing the speciality *during* the 19th century This book is certainly very interesting and at the same time very clearly shows the low standards of that time compared with today New methods by surgeons were often met by jealousy and threats, an opinion held by the well-known Swedish scientist, professor in pathology Robin Fåhraeus of Uppsala, in his book "History of the Art of Medicine" (1944–1950 and 1970)

The major advances in medicine during the 19th century were no doubt the development of anesthesia and bacteriology with antiseptic aseptic techniques. Better hospitals and a better trained nursing staff improved the surgical care of the patients. All these factors have contributed towards better medical as well as surgical treatment.

Thorwald stresses in his conclusions the triumphs accomplished in surgery by researchers from various nations. When this history is written it may be called "Surgery's World Encompassing Empire"

A survey of the development of ear surgery would be deficient without honouring the diligent clerk of Delft, Antony van Leeuwenhoek (1632–1723). He devoted his life to the construction of primitive microscopes which opened up new dimensions to biomedical research Before the use of a microscope with high magnifications, Robert Bárány in Uppsala was doing fenestration-operations, and F R. Nager of Zürich concentrated on the pathology of otosclerosis with great success.

One of the important technical developments in surgery was the use of microscope during an operation, i e microsurgery" The operation microscope was first used experimentally in the spring of 1921 In the autumn of 1921 I tried using a microscope while operating on rabbits. I did these labyrinthine fistulas or fenestrations with a Brinell microscope using 10 to 15 times magnification Engineer N Persson, optician M Storm and I used a monocular microscope and achieved an enlargement of 235 times. I used a primitive binocular microscope in the autumn of 1921 on a case of chronic otitis and of a few cases of pseudofistula symptoms. My chief—Gunnar Holmgren—took the idea a stage further by using the Zeiss stereoscopical microscope at Sabbatsbergs Hospital in Stockholm In spite of extensive use of the microscope it took a rather long time before there was a break through in otomicrosurgery The discovery of various antibiotics seems to have contributed considerably to the development of middle ear surgery

Professor Holmgren developed special operations not only for otosclerosis but also for other diseases within the temporal bone His pioneering work has been continued with great skill by his successor Torsten Skoog. In other

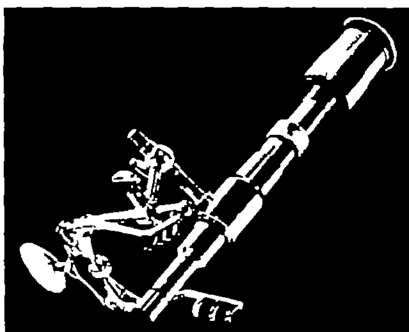


Fig 1 Monocular microscope of C.-O Nylén, N Persson and M. Storm from 1933

parts of Europe the otomicroscope was used by Simson-Hall, Sourdille Cawthorne Huizinga, Jongkees and others. In the United States of America, George E. Shambaugh Jr used a binocular microscope as also did Sullivan, Farrior Guilford and Shea, while Julius Lempert later adopted the technique. Many Scandinavian ENT-specialists started to practise otomicrosurgery such as Andersen, Diamant, Engström Hamberger Herberts, Kristensen Lund, Meurman Palva, Siirala, Stahle. Practicians of otomicrosurgery from other countries were Frenzel, Hasegawa, Hilding House, Lindsay Morimoto Plester Georges and Michel Portmann, Smyth, Wullstein and Zöllner

Besides its use during surgery the microscope is widely used today for inspection and photography of the tympanic membrane and the middle ear The microscope has also been applied to surgery in other parts of the body Thus the microlaryngoscopy and endolaryngeal microscopy described by Kleinsasser has been adopted all over the world. It has also been found useful during hypophysectomy (Angell James) and in replacing the strictured Eustachian Tube with a tympanomaxillary shunt (Drettner & Ekvall). In neurosurgery and vascular surgery finally the operation microscope has achieved widespread application (McO'Brien, Yasargil)

Obviously it is a great pleasure for me to see this very impressive development from an idea that I came upon in 1921 Further improvements are certainly to be expected in the future. Dohlman proposed in his article in 1969- "I believe that many other specialities will in the future profit by microsurgery as much as otomicrosurgery already has" My paper is not so much a detailed story of microsurgery but rather a short review to recall some of the pioneers in otomicrosurgery and some major events in the development of this technique from its advent to the present day It is my sincere hope that this article will be of some use for those wishing to do further research in this interesting field

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Acta Otolaryng 73 455–466 1972

BEHAVIORAL AUDITORY FUNCTION AFTER TRANSECTION OF CROSSED OLIVO-COCHLEAR BUNDLE IN THE CAT

I Pure-tone Threshold and Perceptual Signal-to-noise Ratio

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(Received August 26, 1971)

Abstract. The feline subjects were behaviorally conditioned to respond to pure tone signals at different frequencies based upon the avoidance conditioning procedure. After the transection of crossed olivo-cochlear bundle, the pure tone threshold (250–14 000 Hz) was not altered. Neither was any alteration found in the measurement of the perceptual signal-to-noise ratio at the levels of 30, 50 and 70 dB above the subjects' pure tone thresholds. Morphological confirmation of the brain stem and cochlear end organs demonstrated that proper surgical lesions had been made and there had been subsequent degeneration of the efferent nerve endings around the outer hair cells, especially in the basal coils. It is concluded, therefore, that the absence of the crossed olivo-cochlear bundle has no effect on the behavioral measurement of pure tone thresholds and perceptual signal-to-noise ratio in the cat.

Since the neuroanatomical description of the olivo-cochlear bundle by Rasmussen (1942, 1946, 1960), the course of the bundle and the patterns of peripheral termination within the organ of Corti have been studied in different experimental animal species by many investigators (Engström et al., 1966 Kimura & Wersäll, 1962 Smith, 1967 Spoendlin & Gacek, 1963 Spoendlin, 1968) After stimulation of the efferent fibers, Galambos (1956, 1960) observed that the system suppressed auditory nerve activity He considered the role of the entire system to be inhibitory in nature at least in part.

Although numerous electro-physiological investigations have been conducted, only a limited number of behavioral investigations of this system have been performed previously The study of Dewson (1968) demonstrated the effect upon stimulus discrimination in noise after cutting the olivo-cochlear bundle in the midline in four rhesus monkeys (all cortically ablated except for one subject), he found that their ability to discriminate human vowel speech sounds was impaired in the presence of noise. There was no loss in ability to discriminate the same sounds when noise was absent, or at very low intensity levels. Therefore, he considered that the deficit was related to the perceptual signal-to-noise ratio.

Capps & Ades (1967 1968) studied auditory frequency discrimination after ablating the olivo-cochlear bundle in squirrel monkeys. A modified Wisconsin General Test Apparatus together with a food reward technique was used. Focused ultrasonic irradiation or surgical interruption of the olivo-cochlear bundle in the floor of the fourth ventricle was employed. Following the transection, all animals demonstrated a marked deficit in frequency discrimination performance at 1 000

The present investigation was supported by the National Institute of Neurological Disease and Stroke, Grant NS-08589 and K03-NS-38619

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and 4 000 Hz. They therefore suggested that the efferent auditory system operated to sharpen the frequency resolving power of the inner ear

Trahiotis & Elliott (1970) have recently per formed behavioral studies of effects of sec tioning the crossed olivo-cochlear bundle in cats. Broad band noise was used at levels of 20 30 and 40 dB of masking effect for fre quencies of 500 1 000, and 2 000 Hz. The ex tent of masking effect at 1 000 Hz and 2 000 Hz was found to be increased very slightly after midline section, though the shift was not statistically significant.

The overall objective of the present experiment was to study the physiological and psy chological contribution of the crossed olivo-cochlear efferent system to various aspects of systemic auditory function. The behavioral conditioning approach seemed proper for this purpose in order to measure function in living animal subjects (without anesthesia) under variable acoustic conditions. The morphological results were compared with the data obtained from behavioral conditioning.

Subject

Six adult cats, two females and four males with no otological disorder (based upon otoscopic investigation and confirmation at the time of removal of electronmicroscopic specimens) were utilized for the present investiga tion. Their initial body weight ranged between 6 and $10\,^1/_2$ pounds with a mean weight of $8\,^1/_2$ pounds. Each animal's health condition was also examined and there was no sign of any systemic disease

Screening

Subjects were screened according to the three day 90% criterion (Igarashi & Hoyt, 1963) by avoidance conditioning methods in a cat rotating cage utilizing free field hearing con ditions (in IAC 1202 chamber). Subjects were required to turn the rotating cage within 5 sec of the presentation of a 1 000 Hz, 63.5 dB (re 0 0002 dyne/cm²) pulsed pure tone signal. The signal on–off time was 0.5 sec each. Failure to rotate the cage within 5 sec resulted in a mild electrical shock which was delivered through the cage grids with an intensity ranging between 0.5 to 40 mA and a duration of 0.05 sec. Intensity of the shock was manually controlled depending upon the subject's response body weight and pad skin resistance, etc. A buzzer was sounded when the correct response was performed. A randomized inter val program was employed with a mean inter trial interval of 45 sec with a range of 30–60 sec.

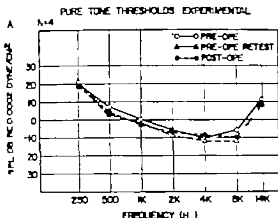
Using the above screening procedure six subjects were selected for further training.

Testing

Pure tone threshold

Screened subjects were trained to respond to several different frequencies namely 250, 500 1 000 2 000, 4 000 8 000 and 14 000 Hz. (14 000 Hz was selected instead of 16 000 Hz because the TV camera which was installed inside the sound proof chamber emitted a frequency of 15 750 Hz.) The absolute pure tone threshold for each frequency was determined by attenuating the signal until the animal no longer responded.

A criterion was used where three negative responses in succession were recorded as no hearing. Two positive responses out of five trials were recorded as hearing equal to the sound pressure level tested. Spontaneous ac tivity was not scored as either positive or negative responses however such activity was counted and recorded. All subjects were tested five days a week, and with two neighboring frequencies a day Since the responses ordinarily became most accurate after two or three testing days, several threshold scores were discarded for this reason. A minimum of four non-discarded scores were needed to calculate a mean threshold at each of the seven frequencies.



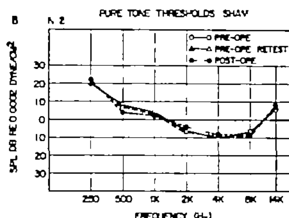


Fig. 1 (A B) Pure tone thresholds for experimental and sham groups, pre-operative (transection of crossed olivo-cochlear bundle) testing, pre-operative retesting, and post-operative testing.

Perceptual signal-to-noise ratio

At each of the seven frequencies tested, pure tone signals with the intensity level of 30, 50 and 70 dB sound pressure level above the subject s pure tone hearing threshold were delivered. Broad band white noise (500–15 000 Hz) was then introduced and its intensity level was gradually increased until the pure tone signal was masked. Noise intensity levels were scored as previously indicated. Inasmuch as the output of the noise generator was not of a constant amplitude the spectrum of white noise between 500 to 15 000 Hz was found to have a 13 dB increase at 14 000 Hz over the amount of white noise at 500 Hz. Since this phenomenon was not known until after the present study was terminated, the effect was not taken into account while calculating the intensity of white noise.

The measurement of perceptual signal to noise ratio consisted of subtracting the dB sound pressure level (re 0 0002 dyne/cm^2) of noise from the dB sound pressure level of the signal. The subjects almost always required a greater dB noise than the pure tone signal. Thus, the ratios were negative numbers. The more negative the perceptual signal-to-noise ratio, the better was the subject able to detect the pure tone signal in a field of white noise.

At least four readings of perceptual signal-to-noise ratio scores were obtained at each of the three intensity levels in the seven frequencies. Due to the standard established in the equipment set up it was not possible to produce noise sound pressure level of sufficient intensity to ordinarily mask frequencies of 50 and 70 dB above the subject s threshold at 250 and 500 Hz.

(C) *Retesting of pure tone threshold and signal-to-noise ratio*

To determine whether the pure tone thresholds had changed after long period of testing, all subjects were retested. Two of the 6 subjects had lower thresholds, one at 8 000 and 14 000 Hz, the other at 8 000 Hz only Their signal-to-noise ratios were retested accordingly to correct for the difference. The overall pure tone thresholds for the 6 subjects did not differ more than 5 dB at each frequency

Surgery (Transection of the crossed olivo-cochlear bundle)

Animals were anesthetized with pentobarbital (40 mg/kg body weight) intraperitoneal injection. An occipital midline incision was made and the occipital bony calvarium was removed by rongeurs to expose the posterior portion of the cerebellum and brain stem. The cerebellum was very gently retracted up-

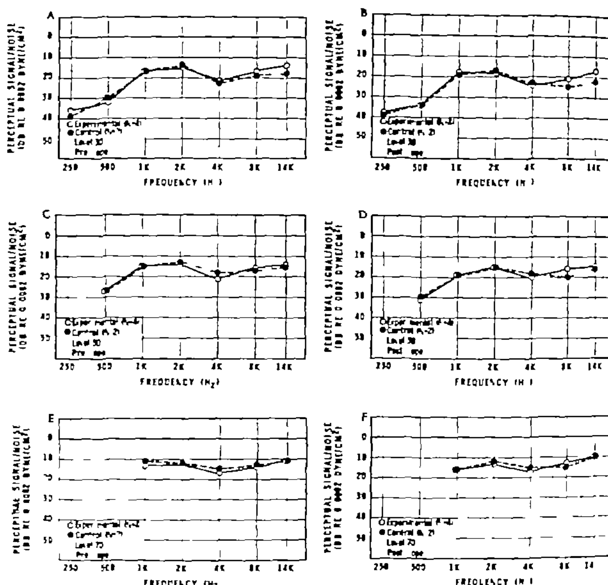


Fig. (A–F) Perceptual signal (30, 50, 70 dB intensity levels) to noise ratios for experimental and sham groups, pre-operative (transection of crossed olivo-cochlear bundle) testing, pre-operative retesting, and post-operative testing.

ward so that a fine pick could be inserted between cerebellum and brain stem. The crossed olivocochlear bundle was sectioned at the level of the facial colliculi in the floor of the fourth ventricle. The sectioning point was calculated by the distance (11 mm) from the obex. A fine hook (2 mm cutting edge) was used to make the lesion. The incision was usually extended about 3 mm both rostrally and caudally from this calculated point; however, in some cases it was intentionally prolonged. Care was taken not to retract the cerebellum too much. All bleeding spots were controlled and the area was covered by a piece of gelfoam. The wound was closed. Two out of 6 cats served as sham operated animals in which the identical surgical procedure was performed except for actual mid-line cutting.

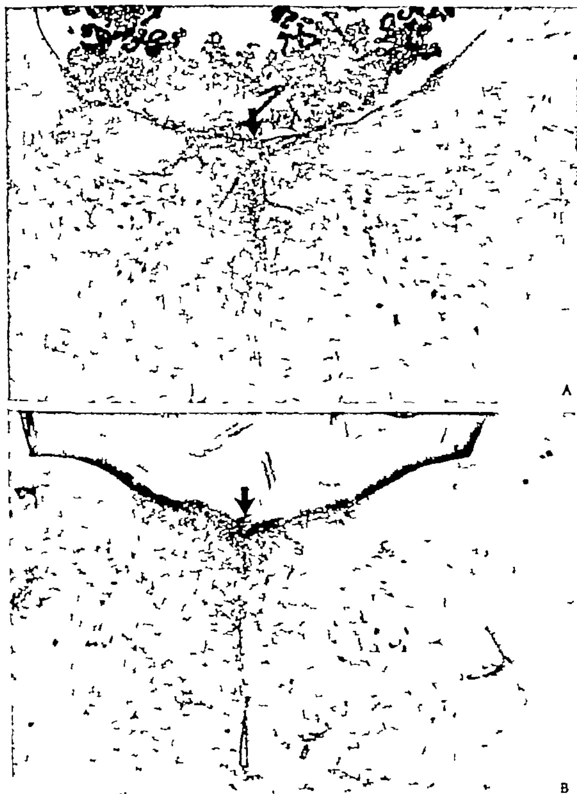


Fig. 3 (*A–B*) Photomicrographs demonstrate the surgical lesions (*arrows*) at the floor of fourth ventricle from two representative (experimental) subjects. (Gallocyanin staining).

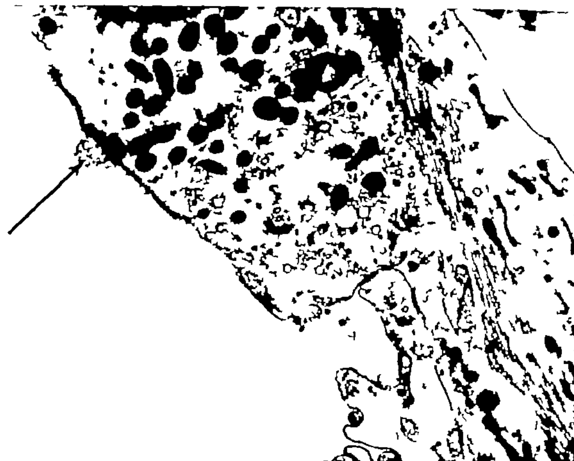


Fig. 4. Electronmicrograph demonstrates the lower portion of first row outer hair cell from the basal turn (about 8 mm from the basal end, chronic cat). Efferent nerve endings are entirely disintegrated and only some cell debris is present (*arrow*). 14 550.

Post-operative testings

All subjects survived the surgical procedure. There was not sign of post-operative infection. Subjects were retested starting about 7 days post-operatively and all were able to rotate the cage competently although some subjects exhibited slight dysequilibrium at the time. The animals were first retested for pure tone threshold, and thereafter for perceptual signal-to-noise ratio, in exactly the same fashion as was done in pre-operative testings.

Morphology

After acquiring sufficient post-operative data, all subjects were sacrificed for morphological investigations. The animals were deeply anesthetized and intravital cardiac perfusion with 2% glutaraldehyde solution, preceded by physiological saline solution, was performed. Cochleas were removed and processed according to the routine procedure of electronmicroscopic preparation. Specimens were embedded in Epon and ultrathin sections were cut by a Porter Blum MT 2 ultramicrotome. Sections were stained with uranyl acetate and lead hydroxide, and were studied under JEM 7 electron microscope. Specimens from at least four different areas of each cochlea were investigated.

The serial cross sections (20 μm) of the brain stem (Gallocyanin staining) were also prepared for neurohistological confirmation of the depth and extent of the surgical lesions.



Fig. 5. Electronmicrograph shows the lower portion of first row outer hair cell from the middle turn (about 17 mm from the basal end, chronic cat). Normal efferent and afferent nerve endings are seen. 9 790.

RESULTS

(A) *Pure tone thresholds*

Fig. 1 (*A* and *B*) demonstrates the averaged pure tone thresholds for the 4 experimental and 2 sham subjects at each of the seven frequencies, including pre-operative testing, pre operative retesting, and the post-operative testing. The data indicate no difference between the pre-operative and the post-operative testing sessions. Essentially the data from sham controls did not differ from those of operated cats. On the other hand, all frequencies except for 250 and 14 000 Hz were slightly improved between pre-operative testing and pre-operative retesting of their thresholds. This slight difference is most probably due to a further practice or training effect. No post-operative effect occurred at the high frequencies such as 4 000 8 000 and 14 000 Hz, which locate the basal turns.

(B) *Perceptual signal-to-noise ratio*

By comparing experimental and sham groups, no significant difference could be determined between pre-operative and post-operative perceptual signal-to-noise ratios at different frequencies and intensity levels (Fig. 2*A*–*F*). As a trend, the post-operative scores were slightly in the direction of better discrimination namely perceptual signal-to-noise ratios were more negative. Again, the possible existence of the training or practice effect should be considered in this regard.

Relatively high negative perceptual signal-to-noise ratios were present at 250 and 500



Fig 6 This electronmicrograph is from the lower portion of second row outer hair cell of the basal turn (about 11 mm from the basal end) from a sham control subject. Normal efferent and afferent nerve endings are seen. Outer spiral fibers (afferent) between Deiters cells are also normal. × 16 000



Fig. 7 Electronmicrograph shows the lower portion of second row outer hair cells from lower basal turn (about 5 mm from the basal end, 13 days post-operative). Degenerated efferent nerve ending can be seen although afferent nerve ending looks normal. Mitochondria have disappeared but some of synaptic vesicles still remain. Subsynaptic cisterna has partly disappeared. 15 390.

Hz because white noise was not present below 500 Hz. In other words, the masking effect of white noise was more pronounced at higher frequencies above 500 Hz.

(C) *Morphology*

The cross serial sections of the brain stem from 4 animals which belong to the experimental group confirmed that both depth and extent (rostral and caudal) of the surgical lesions were proper to eliminate the crossed olivo-cochlear bundle, although the lesions were not clearly distinguishable in these chronic animals (Fig. 3*A*–*B*) when compared with those in acute animals. The rostro-caudal extension of the surgical lesion was found to be 1 0–2.0 mm from the edge of facial genu, (average 2.7 mm from the 11 0 mm point rostral to the obex) The measurements were based upon 15–20% correction which occurs due to nerve tissue shrinkage

From the electronmicroscopic investigation, it was confirmed that many of the efferent terminals to the outer hair cells were eliminated, especially in basal coils (Fig. 4) However rudimentary efferent endings were observed in middle coils and higher (Fig. 5). Sham control subjects did not have any efferent terminal degeneration (Fig. 6). Additional electronmicrographs obtained from



Fig 8. This electronmicrograph is of the first and second row outer hair cells from lower basal turn 4 5 mm from the basal end 13 days post rative). There are three normal-looking afferent re endings below the first row outer hair cell. The efferent nerve ending below the second row outer hair cell is entirely disintegrated and only some cytological debris is still present (*arrow*): however the subsynaptic cisterna along the hair cell membrane appears to be normal. × 11 400.

subjects with short term survival (about 2 weeks) after identical transection of the crossed olivo-cochlear bundle confirmed similar findings namely efferent terminals to the outer hair cells in basal coils and large number of tunnel spiral fibers had disappeared (Figs. 7–9)

DISCUSSION

The testing procedure for perceptual signal-to-noise ratio is a complex one each individual factor was therefore analysed so that no major interference could exist that would affect the experimental purpose

The possible occurrence of a temporary threshold shift from the prolonged exposure to white noise was considered. This possibility was examined by utilizing 3 cats according to the following procedure. First, the pure tone threshold in each frequency was determined in each cat. Then the subject was exposed to white noise (which was described in the present paper) 70 dB level above his own threshold for 15 min the severest condition of the present series of experiments. Immediately after the exposure to the white noise the pure tone threshold acuity was measured once again at each different frequency There was no more than 5 dB threshold change after the noise exposure therefore, the effect from that noise exposure was considered to be negligible

For the measurement of perceptual signal-to-noise ratio higher frequencies were tested

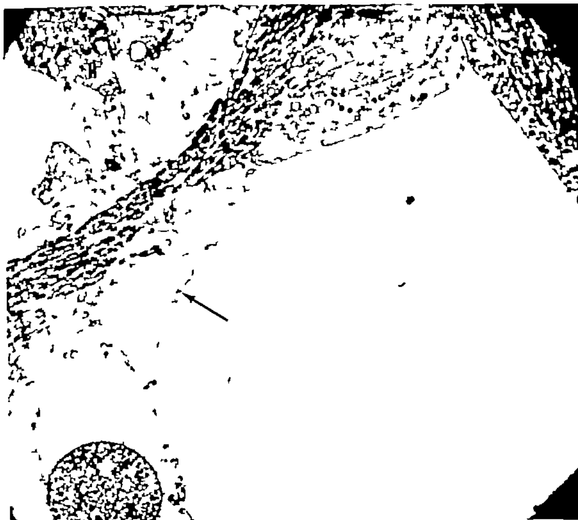


Fig. 9. This electronmicrograph demonstrates almost total disappearance of tunnel spiral fibers (arrow) from the lower basal turn (about 5 mm from the basal end, 12 days post-operative). *H* = inner hair cell. × 9 360.

comparing to the study of Trahiotis & Elliott, with the intent to investigate the basal turn function as the crossed efferent terminals are distributed more in the basal turns. Also higher intensities of pure tone signal and white noise were used, in order to produce more extensive end organ stimulation.

The other concern was the prolonged period of training or practice effect; therefore, before the mid-line operation, pure tone threshold was measured once again after the measurement of perceptual signal-to-noise ratio. In 2 experimental cats, a slight threshold improvement was noticed; the perceptual signal-to-noise ratio was therefore repeatedly measured based on the improved pure tone threshold.

The entire procedure required about 6 months from the initiation of the experiment; however, no cat demonstrated any deterioration in performance during this experiment.

From the present experimental results, it is evident that the morphological elimination of the crossed olivo-cochlear efferent terminals in cats has no effect on the pure tone auditory threshold or perceptual signal-to-

noise ratio. Provided that the crossed olivo-cochlear efferent system was sufficiently activated by the present experimental method, it may be concluded that transection of the crossed olivo-cochlear bundle has no effect upon these two behavioral auditory tasks. On the other hand, because pure tone detection tasks might not be so difficult for cats, the crossed olivo-cochlear efferent system might not have been sufficiently activated although the auditory end organ system was receiving quite intense white noise. Probably in the cat, more difficult and complex tasks are needed to activate the crossed olivo-cochlear system properly or else the translateral olivo-cochlear system has no appreciable contribution to systemic auditory function (Pfalz, 1969). In this regard further experiments are being performed. By stepwise utilization of simple to difficult complex tasks, the ecological validity of the olivo-cochlear efferent system will be systematically investigated.

ACKNOWLEDGMENT

Appreciation is expressed to Mrs M Lewis for her technical assistance

ZUSAMMENFASSUNG

Die Versuchskatzen wurden so trainiert, daß sie im Vermeidungsprozeß auf reine Tonsignale von verschiedenen Frequenzen reagierten. Nach einem Querschnitt des gekreuzten Olivo-Cochlea-Bundels war der reine Ton der Horschwelle (_50–14 000 Hz) nicht verändert. Gleichfalls wurde keine Veränderung in der Bemessung vom wahrnehmbaren Signal zum Geräuschverhältnis vorgefunden bei Stufen von 30, 50 und 70 dB höher als der reine Ton der Hörschwelle der Katzen. Morphologische Bestatigung des Gehirnstammes und des Cochlea-End-Organes bewies, daß richtige chirurgische Verbindungen gemacht wurden, und man konnte die darauffolgende Degeneration der nach außen führenden Nervenenden um die außeren Haarzellen feststellen ganz besonders in den Grundspiralen. Es wurde daher beschlossen, daß die Abwesenheit des gekreuzten Olivo-Cochlea Bündels keinen Einfluß hat auf das Verhalten der Katze bei reinen Ton Hörschwellen und in der Bemessung vom wahrnehmbaren Signal zum Geräuschverhältnis.

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Acta Otolaryng 73 467–475 1972

ULTRASTRUCTURE OF THE MIDDLE EAR MUCOSA IN SECRETORY OTITIS MEDIA

II Mucous Effusion

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(Received June 10 1971)

Abstract Part 2 of an ultrastructural study of the middle ear mucosa from patients with secretory otitis media is presented. Biopsies were taken of the promontorial epithelium of 11 patients with mucous, glue-like effusion in the middle ear By comparison with mucosa from patients with serous effusion, it was found that the cells presumed to secrete serous fluid had disappeared, while the mucous-secreting cells had increased in number Ciliated cells were few and the cilia often degenerated. Many submucous glands were demonstrated. A few specimens showed squamous metaplasia of the mucosa which might present an early stage of a primary cholesteatoma.

Previous light microscopic studies have shown that in secretory otitis media, both experimentally induced and in man, the middle-ear mucosa is characterized by secretory activity containing goblet cells as well as submucous glands (Sala & De Stefani, 1962 Senturia et al. 1962 Bendek, 1963 Friedmann 1963 Sadé 1966) By ultrastructural studies of experimental secretory otitis media Lim & Hussl (1970) has confirmed this secretory stage, while Paparella et al. (1970) found appearances which rather supported the theory of passive transudation. These authors did not divide their materials by the nature of the secretion. We felt that such a division was reasonable. Thereby we hoped to contribute to elucidating whether there are two different diseases or different stages of the same disease. In a previous paper we published the result of an ultrastructural study of the middle-ear mucosa in patients with secretory otitis media in whom operation revealed serous fluid in the middle ear The middle-ear mucosa was found to be in a state of active secretion and showed no signs of pathological degeneration. Below we are submitting the results of studying patients with mucous secretion in the middle ear

MATERIAL AND METHOD

The material was composed of mucosal biopsies from 11 children aged 3–16 years in whom secretory otitis had been diagnosed on the basis of conductive hearing loss and a flat curve in impedance measurement. Most of them had been treated previously by paracentesis or tubulation. The disease had in most cases lasted longer (in the majority of cases for years) than in our previous material in which the middle-ear contents were serous. After paracentesis and removal of the mucous secretion, biopsy was removed with small forceps from the promontorial mucosa, and a tube was inserted into the drum. The specimens were fixed, embedded, stained, and cut as described in the previous paper

RESULTS

In the light microscope the majority of preparations exhibited a two- or t   la

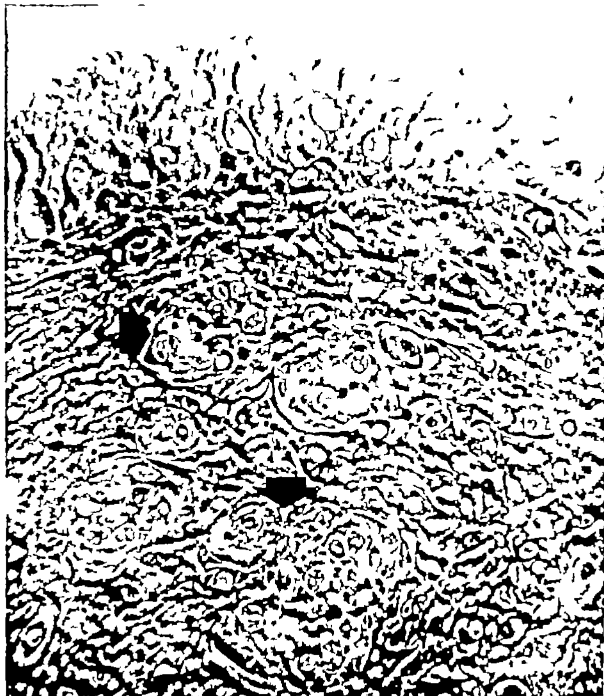


Fig 1 Micrograph of promontorial mucosa from patient with mucous effusion. The epithelium is pseudostratified columnar or cuboidal without cilia. The submucous layer is thickened possessing many glands (*arrows*). 1 645

cuboidal epithelium and the remainder pseudostratified columnar epithelium Ciliated cells were few and often entirely absent Some preparations contained goblet cells of typical shape and several a large number of glands in the thickened submucous layer (Fig 1) In one case the epithelium had undergone metaplasia to squamous epithelium.

Electron microscopy showed fundamentally the same cells as in secretory otitis with serous



Fig. 2. Apocrine secretion into the lumen of a submucous gland. The luminal part of more cells is being expelled into the lumen. The expelled parts contain the normal organelles of the cell including granular reticulum and secretory granules.

effusion apart from the fact that the non-ciliated cell with dark granules was no longer present. As in the light microscope, the ciliated cell had become even less common. Apocrine secretion in the form of bulging of the luminal part of the cell, suggesting pseudopodia, was not observed in any of these preparations. Especially in the glands there was another form of apocrine secretion, giving the impression that the entire luminal part of the cell with its content of organelles was being expelled into the lumen (Fig. 2). Numerous cells bore the marks of their content of "mucigen" granules, i.e. large light granules with or without a central darker core and of extremely varied size (Fig. 3). All cells in the entire depth of the epithelium, apart from the basal cells, might contain these granules which, however increased in frequency towards th face. The cytoplasm of



Fig 3 Luminal part of surface cells dominated by abundant mucigen-like granules of varied size and granular reticulum. Arrows point to granules emptying their content into the lumen.

types: Either pale as in the ciliated, non ciliated and intermediate cells with ample granular reticulum with more or less dilated cisternae a well-developed Golgi apparatus, and mitochondria of varying shape (Fig. 4)

In the other type the greater part of the cytoplasm was occupied by "mucigen" granules, while its remaining part was very dark owing to a densely packed content of granular reticulum with dilated cisternae and ribosomes. In these cells the nucleus was always pyknotic. As already mentioned, the ciliated cells were few and in several cases there were cells having degenerated cilia. In a few instances the ciliated cells also contained large round structures enveloped by a membrane of a kind similar to the "mucigen" granules without a dark core (Fig. 5) In some cells there were signs of brisk regeneration of the cilia with numerous basal bodies beneath the surface (Fig. 6 A, B). The lumina of the submucous glands were moulded with secretory granules which had fused, to a major or minor extent, into a homogeneous substance and with the previously described membrane-enveloped desquamations of luminal cellular segments with a content of diverse organelles.

DISCUSSION

Compared with previously described findings in the middle-ear mucosa from patients with serous effusion, it was found that in the mucous form of secretory otitis the epithelium had altered, in part quantitatively towards greater secretory activity and in part presumably qualitatively the cells with dark granules presumed to secrete serous fluid having dis-



Fig. 4. Two types of cell containing mucigen-like granules are demonstrated. Arrows point to the dark type with many granules and a sparse dark cytoplasm marked by a well developed Golgi apparatus and granular reticulum both with dilated cisternae. The other cells are of the pale type also containing mucigen-like granules. The light areas with few cell organelles ( ) are the result of apocrine secretion as shown in Fig. 2.

appeared and the cells presumed to secrete mucus having increased in number and distribution throughout the entire height of the epithelium. Moreover it seemed that all cells, except the basal ones, might exhibit secretory activity and the majority of the ciliated cells had lost their cilia and had presumably become secretory. The pseudopodia-like apocrine secretion had been replaced by apocrine secretion in which the entire luminal part of the

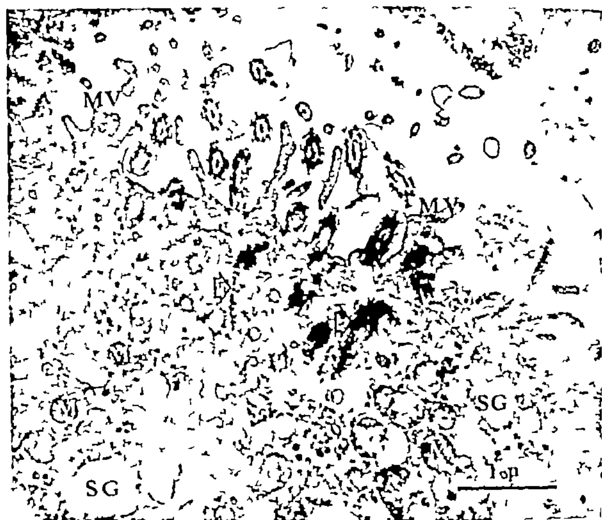


Fig. 5. Ciliated cell with membrane bounded structures (*SG*) similar to the secretory granules of the non-ciliated cell. The cilia with basal bodies and cross-striated rootlets (*arrows*) are well visualized. *M* mitochondria. *MV* microvilli.

cell was expelled. The specimen from one patient of this group (as well as from one patient of the group with serous effusion) showed squamous epithelium.

A few previous authors (Bendek 1963 Paparella, 1964) have found a common occurrence of squamous metaplasia of the middle-ear mucosa in secretory otitis. In the present material it was not possible to decide whether this finding represents metaplasia of the middle-ear mucosa into squamous epithelium as often seen in chronic suppurative otitis media, or whether it represents the lateral aspect of a piece of tympanic membrane which has been removed by accident at the same time as the mucosal biopsies. If the finding does really represent metaplasia of the mucosa behind the intact drum we have at least part of the explanation of the pathogenesis of primary cholesteatoma.

In one case of secretory otitis media, Sadé (1966) found a gland which was PAS-negative He therefore assumed that the serous secretion in these patients formed in separate serous glandular elements. Hussl & Lim (1969), in an ultrastructural and cytochemical study of the guinea-pig middle-ear mucosa, found 3 types of cell. Goblet cells with large, pale,

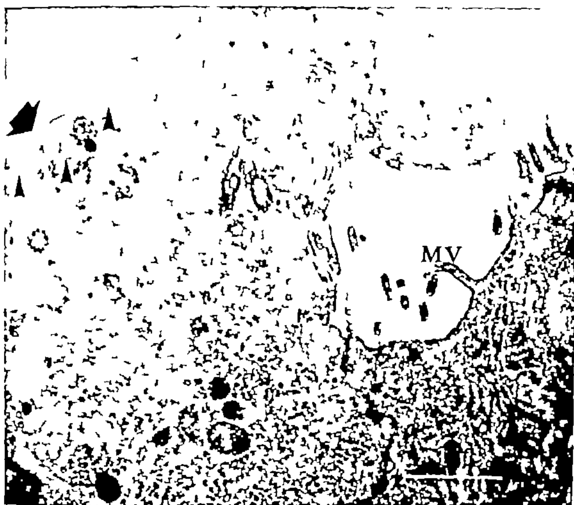


Fig. 6 (A) Luminal part of ciliated cell with many basal bodies subjacent to cell surface (*small arrows*) indicating brisk generation of cilia. (*MV*) microvilli

mucigen granules, in some instances with a darker core, intermediary cells containing pale as well as darker granules, and finally a dark granulated cell. The cytochemical study showed that the goblet cells fell into the sero-mucous category according to the classification of Shackleford & Klapper (1962) containing neutral as well as acid mucopolysaccharides, while the intermediary cells containing only small amounts of mucopolysaccharides and the dark granulated cells containing no mucopolysaccharides fell into the serous (non-mucous) category. In a later study (1969) of the human middle-ear epithelium the dark granulated cell was not found but the authors still felt in a position to differentiate two types of cell, viz. intermediary which in the cytochemical study proved non mucous (serous) in nature and the goblet cell which was sero-mucous. It was emphasized however that the diverse ultrastructural and cytochemical characteristics of these secreting cells might be expressions of different phenotypes of the same secreting cell. On the basis of an ultrastructural study of the mucosa in the different parts of the human middle ear (Hentzer 1970) we are inclined to believe that the la sumption is correct. However in

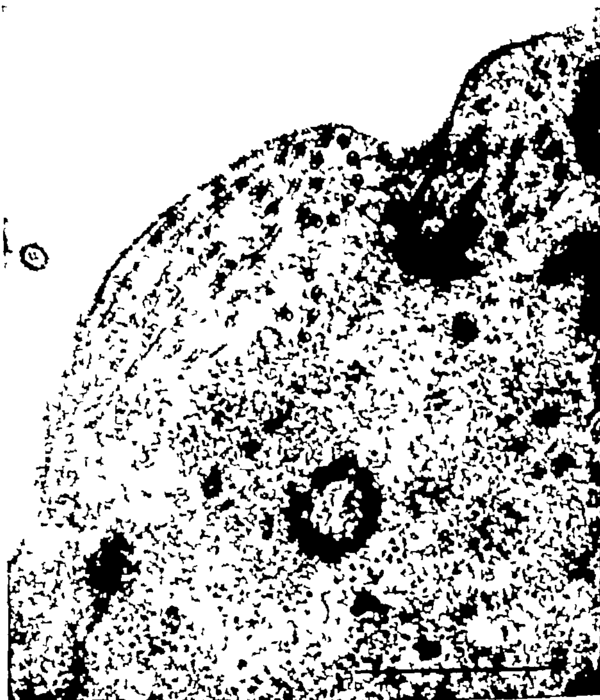


Fig 6. (B) Close up of area indicated by big arrow on Fig. 6 A. Cross section of the basal body shows nine peripheral fibres with "figure-of-eight" appearance (*arrows*) and two central fibres.

radiographic investigation of the normal middle-ear mucosa of the guinea-pig, Lim (1970) demonstrated that after administration of tritium-labelled leucine there was a large uptake of amino acids in the transitional area of the middle ear where dark granulated and intermediary cells predominate, indicating that these cells were of serous type, while the

mucus-secreting goblet cells of the Eustachian tube incorporated only negligible amounts of protein.

The result of our studies accords with Lim and Hussl's in so far as the dark granulated cell was found only in the epithelium from patients with serous effusion. On the other hand, the number of these dark granulated cells in serous effusion was small (even smaller than in the normal middle-ear mucosa), and the number of cells having light mature granules of the kind present in the goblet cell was large. Thus, summing up our ultrastructural studies of the mucosa in the normal middle ear and in the middle ear of patients with serous and with mucous secretion it may be said that the epithelium in the three materials did not exhibit major qualitative differences. On the other hand, there were quantitative differences, patients with mucous secretion *showing the most marked secretory* activity all cells apart from the basal cells exhibiting signs of secretion. Even the ciliated cell loses its cilia and is presumably transformed into a secretory cell. Here we have a condition in which the normal balance between a small number of secretory cells and a large number of ciliated ones, which can transport the formed secretion away from the middle ear has changed into an extremely large number of secretory cells which produce secretion into a small cavity with a poor outflow and few ciliated cells.

ZUSAMMENFASSUNG

Der zweite Teil einer elektronenmikroskopischen Untersuchung der Mittelohrschleimhaut bei serösem Mittelohrkatarrh mit mucösem Exudat ist gegeben. Die Präparate wurde aus der Promontorialschleimhaut von 11 Patienten mit mucösem, „glue" Exudat im Mittelohr genommen. Im Vergleich zu der Schleimhaut von Patienten mit serösem Exudat gab es keine neuen Zellen sondern eine grossere Menge von Zellen mit Mucigen Granula. Die ziliaren Zellen waren nur wenig und die Zilien oft degeneriert. In der Submucosa befanden sich viele Drüsen. In einzelnen Präparaten war das Epithel zu mehrschichtigem Plattenepithel metaplasiert. Dies könnte ein frühes Stadium des primären Cholesteatomes repräsentieren.

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Acta Otolaryng 73 476–483 1972

ETHACRYNIC ACID-INDUCED HEARING LOSS IN GUINEA PIGS

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(Received July 16 1971)

Abstract In normal guinea pigs Ethacrynic acid was shown to cause a reversible high-tone hearing loss with evidence of recruitment. There was a definite tendency to a dose-response relationship. The unchanged Preyer reflex level, in animals with even marked elevations of the hearing threshold, demonstrated the impossibility of evaluating hearing function by Preyer reflex determinations only. Kanamycin-treated animals demonstrated unimpaired ability to respond to Ethacrynic acid as above.

Cochlear degenerative processes in animals have attracted the attention of a large number of investigators. Oto-toxic substances, mainly several aminoglucoside antibiotics, have frequently been used. A recent product with a conceivable ototoxic side effect is Ethacrynic acid, a potent diuretic. Several reports have appeared on reversible and irreversible hearing impairments in uremic patients treated with Ethacrynic acid only or in combination with known ototoxic antibiotics (Irons et al. 1965 Maher & Schreiner 1965 Schneider & Becker 1966 Schmidt & Friedman 1967 Matz & Naunton 1968 Hanzelik & Peppercorn 1969 Mathog & Klein 1969 Matz et al. 1969 Ng et al. 1969 Pillay et al., 1969 Council on Drugs, 1969).

Hearing threshold tests on animals treated with Ethacrynic acid have not been previously published. The aim of the present investigation was to elucidate some properties of Ethacrynic acid-induced hearing loss in normal and in Kanamycin-treated guinea pigs

MATERIAL AND METHOD

Six healthy adult guinea pigs were used.

The animals were conditioned for determination of hearing threshold as described by Anderson & Wedenberg (1965) The animals showed thresholds of hearing within normal limits for the frequencies used. 125 250 and 500 Hz, 1 1 $^1/_2$ 2, 3 4 8 and 12 kHz. The Preyer reflex threshold was recorded at $^1/_2$, 1 2, 3 and 4 kHz and was also found to conform to normal values.

Five guinea pigs were each given Ethacrynic acid intraperitoneally in doses amounting to 2, 4 and 8 mg/kg body weight in separate tests. Audiograms and Preyer reflex thresholds were obtained 1 $^1/_2$, 3 4 $^1/_2$ 6 and, if required, 24 hours after each injection. To avoid cumulative effects an interval of several days was made between the experiments.

Two guinea pigs were given Kanamycin sulphate intraperitoneally 400 mg/kg body weight once daily for 5 days. Seventeen days after the last injection the animals received 8 mg/kg Ethacrynic acid intraperitoneally and hearing functions were ascertained as above

Kanamycin sulphate 400 mg/kg body weight was administered intraperitoneally to another animal for 2 days. Ethacrynic acid 8 mg/kg body weight was given intraperitoneally immediately after the second Kanamycin injection and hearing tests were performed.

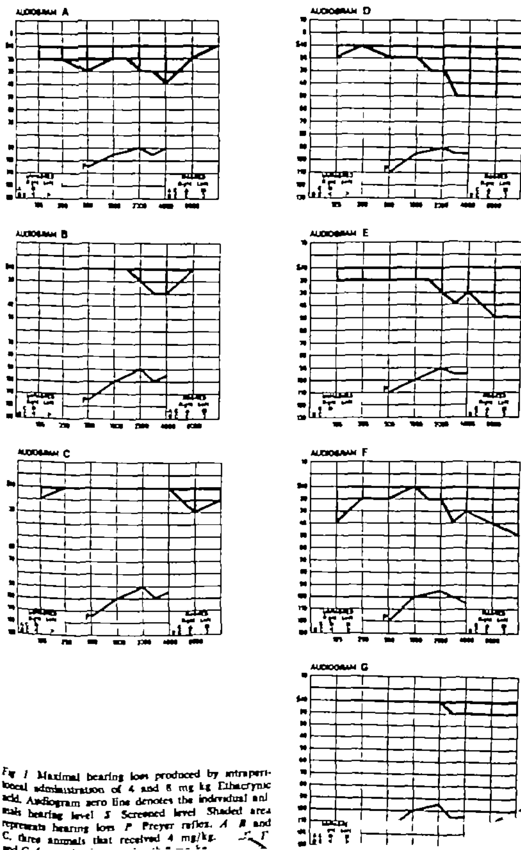


Fig 1 Maximal hearing loss produced by intraperitoneal administration of 4 and 8 mg kg Ethacrynic acid. Audiogram zero line denotes the individual animals hearing level *S* Screened level. Shaded area represents hearing loss *P* Preyer reflex. *A*, *B* and C, three animals that received 4 mg/kg.

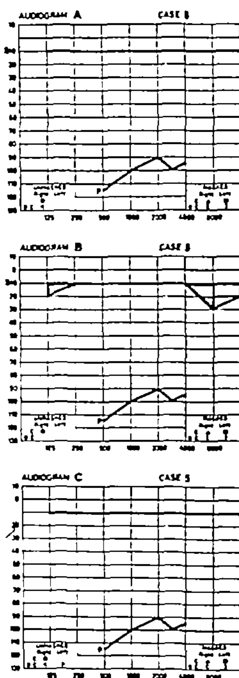


Fig 2 Course of Ethacrynic acid-induced hearing loss, 4 mg kg, guinea pig no. 5 Audiograms and symbols as in Fig. 1 Hearing functions after 1 hours (*A*), after 3 hours (*B*) and after 4 hours (*C*).

RESULTS

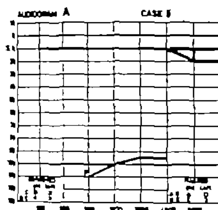
No change in hearing thresholds were obtained with a dose of 2 mg/kg of Ethacrynic acid. The higher doses, however invariably caused a reversible hearing loss, that tended to be less and of shorter duration with 4 mg/kg than with 8 mg/kg (Fig. 1). For both doses maximum hearing loss occurred at about the same time, around 3 hours. Return to normal hearing was observed after $4^1/_2$–24 hours. However a rather marked individual variation was observed in the magnitude of the hearing impairment

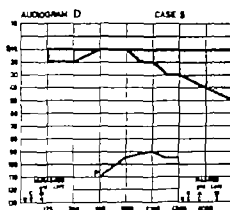
Details of the course in one animal are shown in Figs. 2 and 3 The main effect on the hearing threshold occurred invariably in the higher frequencies. At the higher dosage, however even the lower frequencies tended to be affected. The Preyer reflex thresholds remained unchanged despite even a pronounced hearing threshold elevation.

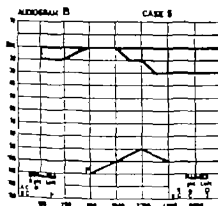
The two guinea pigs that received Kanamycin sulphate for 5 days showed quite divergent reactions. On the third day after the last injection both animals presented a moderate high frequency hearing threshold loss, however in one animal the Preyer reflex threshold remained normal whereas in the other a marked rise occurred. The animal (*case 19*) *with the normal Preyer reflex* level showed, on the fifth day after Kanamycin treatment a pronounced high frequency loss. On the 12th day however hearing was restored to normal (Fig. 4 case 19). The other animal (case 1036) with an early rise of the Preyer reflex threshold, exhibited a progressive hearing threshold impairment affecting mainly the high frequencies. The Preyer reflex could not be elicited after 17 days (Fig. 4 case 1036).

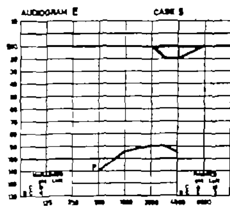
Both these animals still reacted to Ethacrynic acid, 8 mg/kg at 17 days after completed Kanamycin treatment (Fig. *5*) In case 19 the response was marked, in case 1036 of a lesser magnitude.

The guinea pig (case 6) that received Kanamycin for 2 days did not exhibit any impairment in its hearing functions. Immediate Ethacrynic acid administration 8 mg/kg, caused a reversible high-frequency hearing loss (Fig 5) previous values were restored









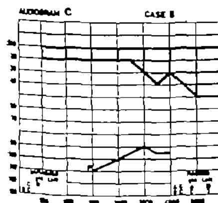


Fig. 5. Course of Ethacrynic acid-induced hearing loss, 8 mg/kg, guinea pig no. 5. Audiograms and symbols as in Fig. 1. Hearing functions after 1 / hours (*A*), after 3 hours (*B*), after 4 / hours (*C*), after 6 hours (*D*) and after 24 hours (*E*).

within 24 hours. The Preyer reflex threshold remained unchanged throughout the course.

DISCUSSION

A single dose of Ethacrynic acid, 4 mg/kg body weight or more, caused reversible impairment of hearing. The characteristic response was an elevation of the hearing threshold, most pronounced in the high frequency area. The Preyer reflex threshold remained at normal level. There was a definite tendency towards a dose-response r ship. Small doses caused a lesser d hearing and of shorter duration

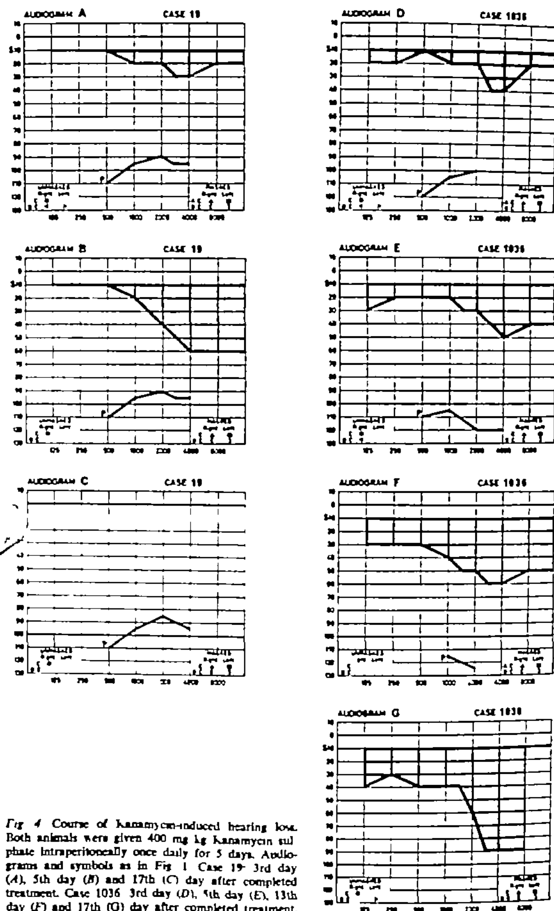


Fig 4 Course of Kanamycin-induced hearing loss. Both animals were given 400 mg kg Kanamycin sulphate intraperitoneally once daily for 5 days. Audiograms and symbols as in Fig 1 Case 19 3rd day (*A*), 5th day (*B*) and 17th (*C*) day after completed treatment. Case 1036 3rd day (*D*), 5th day (*E*), 13th day (*F*) and 17th (*G*) day after completed treatment.

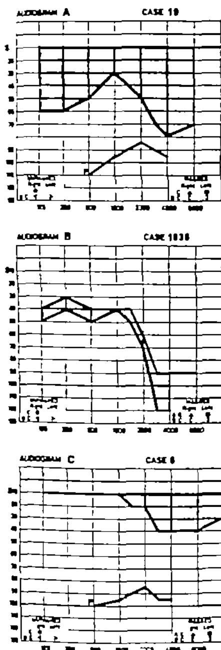


Fig 5 Maximal hearing loss produced by 8 mg kg Ethacrynic acid in guinea pigs treated with Kanamycin. Audiograms and symbols as in Fig 1 (*A*) Animal 19 (cf. Fig. 4). (*B*) Animal 1036 (cf. Fig. 4) Both animals received Ethacrynic acid 17 days after completed Kanamycin treatment. (*C*) Animal 6, which received Ethacrynic acid immediately after 2 days of Kanamycin treatment.

dividual variations, possibly due to differ encies in susceptibility were marked.

Anderson & Wedenberg (1965) demonstrated that the Preyer reflex in guinea pigs behaved very similar to the stapedius reflex in man. Such a condition would be expected with regard to the anatomy and physiology of the pathway of the Preyer reflex arc in guinea pigs (Totsuka et al. 1954). An artificial conduction hearing loss by plugging the animals ear canals caused a parallel elevation of hearing threshold and Preyer reflex threshold. Cochlear damage, such as from intense acoustic trauma, caused in the guinea pigs a high-tone hearing threshold loss, with unchanged threshold level of the Preyer reflex. Furthermore simultaneous recordings of the middle ear muscles reflex and the Preyer reflex thresholds in guinea pigs have shown the threshold levels to coincide, in animals with normal as well as elevated reflex thresholds (Ernstson, in press). This relation between hearing and reflex thresholds has been extensively studied in man (Metz, 1952, Kristensen & Jepsen, 1952, Thomsen, 1955) and the vast knowledge accumulated may now be transferred to studies on hearing functions in guinea pigs.

Thus in the present investigation the unchanged Preyer reflex level despite a marked hearing-threshold impairment, indicated the presence of recruitment. Consequently the effect of Ethacrynic acid on hearing takes place in the cochlea. Furthermore, these experiments demonstrate the pitfalls that occur if the Preyer reflex alone is used to evaluate the hearing function, as the presence of recruitment will invariably distort the results.

The disparity in the reaction to Kanamycin was striking. The same dose caused in one animal a reversible hearing loss, though with a protracted course, whereas the other animal demonstrated a slowly progressive hearing impairment. Both animals, however preserved their ability to react to Ethacry acid. In case 1036 the response than in case 19 For both animals,

the previous levels of hearing were regained well within 24 hours after administration of the Ethacrynic acid. The animal that received Ethacrynic acid immediately after the second Kanamycin injection also responded with a reversible hearing loss

Stupp (1970) in his report on the ototoxicity of Kanamycin and other antibiotics, produced evidence that membrane systems in the cochlea were damaged prior to or concomitantly with hair cell degeneration Possibly Ethacrynic acid does not act on the same membrane or enzyme system as Kanamycin

The individual variation in response to Ethacrynic acid and Kanamycin are intriguing The animals were adults with normal hearing uniform bel [illegible] and without visible signs of disea[illegible] [illegible]e error in administration would [illegible] [illegible]nt to account for the Kanamycin [illegible] [illegible]ver differences in renal fu[illegible] [illegible]sm or cochlear vulnerability [illegible] [illegible]or the variations observed. Th[illegible] [illegible]may reflect the individual anim[illegible] [illegible]tem.

Quick & Du[illegible] [illegible] reported an electron microsco[illegible] [illegible]f cochleae from guinea pig[illegible] [illegible]acrynic acid. Very high intra[illegible] [illegible]xceeding 30 mg/kg body we[illegible] [illegible]d sapp arance of Preyer reflex [illegible] [illegible]n the stria vascularis were observed [illegible] high doses but with 10–15 mg/kg Ethacrynic acid no consistent anomalies occurred In cats Mathog et al. (1970) found changes of cochlear microphonics and action potentials with intravenous doses of 10 mg/kg Ethacrynic acid. Kohonen et al. (1970) using guinea pigs, observed similar changes with 20 mg/kg Ethacrynic acid.

As the hearing threshold impairment caused by Ethacrynic acid (4 and 8 mg/kg i.p.) was always reversible morphologic changes would not be expected.

The study confirms that the Preyer reflex test cannot be used as a sole hearing test In recruiting lesions a marked hearing threshold loss may very well be present in spite of a completely normal reflex threshold.

ZUSAMMENFASSUNG

Bei normalen Meerschweinchen konnte gezeigt werden, dass Ethacrynsäure einen reversiblen Hörverlust für hochfrequente Töne mit dem Kennzeichen des Recruitment hervorruft. Eine Tendenz zu einer dosisabhängigen Beziehung für den Hörverlust wurde deutlich. Das unveränderte Preyer Reflex-Niveau auch bei Tieren mit markanten Erhöhungen der Hörschwelle zeigt, dass es unmöglich ist, die Hörfunktion ausschliesslich durch das Bestimmung des Preyer-Reflexes zu untersuchen. Kanamycin-behandelte Tiere zeigten eine unbeeinflusste Fähigkeit auf Ethacrynsäure zu reagieren.

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Acta Otolaryng 73 484–492, 1972

COCHLEO-VESTIBULAR INVOLVEMENT IN OTOSCLEROSIS

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(Received August 25 1971)

Abstract. A clinical analysis was carried out upon 70 otosclerotic patients. As the age of patients increased there was a parallel increase of deafness and the latter was accompanied by a greater cochleo-vestibular involvement. Those ears with cochlear involvement showed a greater percentage of caloric abnormalities than the ears with a purely conductive deafness. Fifty-six patients (77 7) had vestibular symptomatology and in this group there was no correlation between either age deafness or severity of symptoms. *It is concluded that in the course of time* the otosclerotic lesions cause a progressive involvement of both cochlear and vestibular capsules thus producing lesions of the sensori-neural elements in the anterior and posterior labyrinths. The vestibular involvement may explain the presence of vertiginous crises in at least some of the patients.

The topic of cochleo-vestibular involvement has been the subject of a number of papers dealing with otosclerosis nevertheless it is still by no means clear to what extent the disease is responsible for the cochleo-vestibular damage which has been observed in certain cases and what is the pathogenesis of this damage. The purpose of the present paper is to make some contribution to the study of the cochleo-vestibular involvement in otosclerosis through the analysis of a variety of clinical observations.

Cochlear involvement in otosclerosis has been studied in its clinical and histopathological aspects. Upon the basis of clinical investigations, Meurman & Wolff (1960) found perceptive involvement in 24 5% of a group of otosclerotic patients, Glorig & Gallo (1962) in a comparative study between otosclerotic patients and the general population in the same age groups, found that the perceptive component of the deafness in otosclerotic patients older than 60 years was much greater than that found in non-otosclerotic subjects; Carhart (1966) in an analysis of a group of more than one thousand subjects diagnosed as sensori-neural deafness of unknown etiology" concludes that an important number of them seemed to correspond to what he called cochlear otosclerosis" the characteristics of this entity are (*a*) it appears at an early age, generally before the person is 45 years of age (*b*) subjects may have antecedents with familial deafness (*c*) it is generally symmetrical, and (*d*) of gradual onset. He adds that usually it is accompanied by very good speech discrimination, positive recruitment high S.I.S.I. scores and Type II Békésy tracings.

As to the histopathology of temporal bones of patients with otosclerosis Nager & Fraser (1938) and Rüedi (1963) have shown that in advanced cases, new lamellar bone may invade the inner ear especially the tympanic scala of the basal coil Wolff (1950) found atrophy of the spiral ganglion and organ of Corti in the basal coil of the cochlea in otosclerotic temporal bones Nager (1966) was able to observe extensive and diffuse otosclerotic foci in the cochlear and vestibular capsules connected with alterations of the spiral ligament, stria vascularis, *Corti's organ* (absence of hair cells), nerve fibers and spiral

ganglion cells. Benitez & Schuknecht (1962), found that when the otosclerotic foci involve the otic capsule as far as the endosteum of the cochlea it gives rise to atrophic changes of the spiral ligament in all the three coils of the cochlea.

Other authors have correlated the clinical findings with histopathological studies. Rüedi (1965), found a clear-cut correlation between the extent of the spiral ligament in contact with the otosclerotic focus and the severity of the perceptive deafness while Schuknecht & Gross (1966), and Lindsay & Beal (1966), have reported atrophic changes in the area of the spiral ligament adjacent to the otosclerotic focus. These areas seem to be connected with the presence of a greater perceptive component of the deafness. Rüedi & Spoendlin (1966), found circumscribed stria vascularis changes (a greater number of enlarged capillaries together with a hypertrophy of the epithelium of the stria) where an otosclerotic focus was in contact with the connective tissue of the spiral ligament. These stria vascularis changes were observed in some cases of severe perceptive deafness.

Studies concerned with vestibular involvement in otosclerosis are less numerous than those concerned with cochlear involvement, nevertheless a number have appeared, based largely upon clinical observations. Some authors have analysed the subjective manifestations of vestibular damage. Rasmussen (1949) found that 27% of his otosclerotic patients had vertigo prior to the operation. Hulk & Jongkees (1950) reported that 30% of a group of otosclerotic subjects complained of giddiness or imbalance prior to the operation. Cawthorne (1955) in an analysis of two thousand otosclerotic patients, found that 24% of them had vestibular symptoms. Felisati (1958), reports that 30% of his patients had moderate vertigo. Reinecken (1960) found that 25% of his otosclerotic patients complained of vertigo before the operation and Profazio et al. (1963), found vestibular symptoms in 26% of their cases.

Other authors have studied vestibular impairment objectively in terms of the presence of spontaneous nystagmus or caloric test abnormalities. Fisch (1965), carried out an electronystagmographic investigation of vestibular function in a group of otosclerotic patients and found that 28.8% of them had spontaneous or positional nystagmus, in addition he noted that those subjects with vestibular abnormalities showed evidence of cochlear dysfunction in contrast to those patients with absent vestibular abnormalities. Bagnarol & Cavallari (1968) found caloric test abnormalities (usually a slight canal paresis) in 56% of their patients. Recently Meurman et al. (1969), in an electronystagmographic study of a group of otosclerotic subjects state that 35% of them had positional nystagmus or caloric test abnormalities.

MATERIAL AND RESULTS

The present study is concerned with a clinical analysis of 202 otosclerotic patients (404 ears) examined over a period of 2 years, 59 (29.2%) of these were males and 143 (70.8%) were females. The scattergram shown in Fig. 1 relates the average hearing levels of each ear at the three frequencies 500, 1 000 and 2 000 Hz to the age of the patients. Although no attempt has been made to determine the regression curve it is clear that there is a

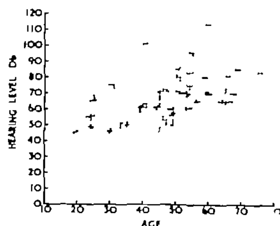


Fig. 1

Table I *Relationship of deafness to age and perceptive involvement*

Clinical group	No. of ears	Average age	Perceptive involvement
A	56 (14)	37	11/56 (?0)
B	288 (71)	47	27/288 (9%)
C	60 (15)	53	28/60 (47%)

progressive increase in deafness with age This relationship emerges more clearly in Table I

Here the ears have been classified into three clinical groups according to the average hearing levels A) with deafness less than 40 dB 56 (14%) B) with deafness between 40 and 80 dB 288 (71%) C) with deafness greater than 80 dB 60 (15%). The average age in each group is 37 47 and 53 respectively In column three are shown the number of ears in each group in which sensory neural involvement has been established the percentages refer to the total numbers of ears in each group It will be seen that much the greatest involvement occurs in group C and it would seem reasonable to conclude there[illegible]ore that the increase in the age of the patient [illegible] magnitude of the deafness is accompanied by greater cochlear involvement.

Evidence of vestibular involvement was derived from the subjective vestibular symptoms (vertigo imbalance etc.) and from the results of the caloric tests carried out according to the Fitzgerald and Hallpike technique. Caloric abnormalities were considered to be present when there was a difference of more than 15 sec in the duration of the nystagmus at the two ears.

One hundred and forty-six patients (i.e. 72.3% of the total) had no subjective vestibular symptoms, of these 54 (37%) were males and 92 (63%) females. An analysis of this group with respect to their hearing levels within the clinical groups A, B and C specified above and the caloric test results is shown in Table II

The percentages shown in column one refer to the total number of ears in each clinical group and it will be seen that the percentage number of patients remaining free from vestibular symptoms remains remarkably constant in the groups A, B and C at 77% 70% and 78% respectively despite the increase in hearing loss. In column two are shown the numbers and percentages of ears with caloric abnormalities in each group The denominators refer to the number of ears upon which caloric tests were available. By contrast to column one it will be seen that there is a progressive increase in the percentage number of ears with caloric abnormalities from 9% in group A to 10% in group B to 36% in group C. In other words a significant proportion of the patients free from vestibular symptoms with deafness in excess of 80 dB do in fact present with caloric abnormalities. The reason for this is not immediately clear however in view of the fact that we have already established a correlation between age and deafness it is to be expected that the patients in this group were of the older age group No statistical analysis appears to have been made of the distribution of caloric patterns with respect to age in normal subjects and it may well be that the abnormalities found here are no more

Table II *Patients without vestibular symptoms. Relationship between deafness caloric abnormalities and perceptive involvement*

Clinical group	No. of ears	Caloric abnormalities	Perceptive involvement
A	43/56 (77)	3/33 (9%)	10 (23%)
B	202/288 (70)	16/156 (10)	19 (9%)
C	47/60 (73)	12/33 (36%)	22 (47%)

Table III *Patients with vestibular symptoms. Relationship between deafness, caloric abnormalities and perceptive involvement*

Clinical group	No. of ears	Caloric abnormalities	Perceptive involvement
A	13/56 (23%)	?/9 (22%)	1 (8%)
B	86/288 (30)	15/76 (20)	8 (9%)
C	13/60 (22%)	8/11 (73%)	6 (46%)

than those to be expected in normal subjects within this age group. On the other hand, as will be seen from column three which gives the numbers and percentages with perceptive involvement it would seem to be not without significance that the percentage and number in group C is significantly increased at 47% This suggests that cochlear involvement is accompanied by vestibular involvement whether or not vestibular symptoms are present and we will return to this point later

Let us now turn to the patients with vestibular symptoms. These numbered 56 in all, that is to say 27 7% of the total. Of these, 51 (91%) were females and only 5 (9%) males. A similar analysis to that carried out upon the patients without vestibular symptoms is shown in Table III where it will be seen, as is to be expected, that the percentage number of patients within each group with caloric abnormalities is much increased with respect to the patients without vestibular symptoms. Perhaps the most striking finding is the high incidence of patients with caloric abnormalities in the group with deafness greater than 80 dB namely 73% This is more than double the figure for the same group of patients without vestibular symptoms. By contrast, the figures given in column three show the numbers and percentages with perceptive involvement and it will be seen that these are remarkably similar to those of the group without vestibular symptoms. It would seem, therefore, that vestibular involvement may occur without the cochlea necessarily becoming involved.

The next point of enquiry concerns the correlation of the severity of the symptoms with caloric abnormalities. For this purpose the severity of symptoms was graded as follows:

Grade 1 Those subjects who had slight giddiness (without a true subjective or objective rotatory sensation) sometimes accompanied by mild imbalance. 21 37%

Grade 2 Those patients who occasionally had a transitory bout of vertigo (subjective or objective rotatory sensation) brought on by head movements and sometimes accompanied by moderate imbalance 11 20%

Grade 3 Subjects with spontaneous vertiginous crises not related to head movement, generally paroxysmal, recurrent and accompanied by nausea (sometimes vomiting) and severe loss of balance. 24 43%

Table IV *Relationship of severity of symptoms and caloric abnormalities*

Grade	No. of ears	Caloric abnormalities
1	42	5/34 (15%)
2	22	5/22 (23)
3	48	15 40 (38%)

The analysis is shown in Table IV and it will be seen that with the increase in severity of symptoms there is a concomitant increase in the percentage number of subjects with caloric abnormalities rising from 15% to 23% to 38% In view of the severity of the symptoms in Grade 3 it might be thought that the latter figure is perhaps somewhat low However the possibility does exist that the vestibular lesion in many of these patients may have been utricular in origin and in such subjects caloric patterns are typically normal. However reference has earlier been made to the apparent resistance of the males in our group to develop vestibular symptoms. In fact, of the total number of patients only 5 out of 59 males (i.e. 8.5%) developed vestibular symptoms in contrast to 51 out of 143 (35 6%) females. One cannot exclude, therefore the possibility that female neurosis may be a contributory factor

Table V relates the three grades of vestibular

Table V *Relationship of severity of symptoms to deafness*

Grade	A	B	C
1	6	32	4
2	2	18	2
3	5	36	7

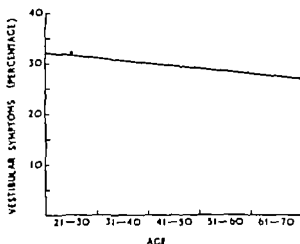


Fig 2

symptoms to deafness. No clear correlation emerges and it would seem that the severity of the symptoms is not related in any way to the hearing loss.

The correlation between age and incidence of vestibular symptoms is shown in Fig. 2. Although the incidence appears to be higher in the younger age group this is unlikely to be significant and by and large it would seem that the incidence of patients complaining of vestibular symptoms is reasonably constant at ages. It follows that the symptoms are likely be related to the disease process and not to ther factors connected with ageing.

Finally Table VI shows the correlation between both perceptive and vestibular involvement. Thus, of the ears without perceptive involvement 15% of those upon whom caloric tests were carried out as indicated by the denominators showed abnormalities. By contrast 33% of the patients with perceptive involvement upon whom caloric tests were carried out showed abnormalities. This is a finding of some interest because it follows that cochlear involvement is not necessarily accompanied by vestibular involvement. On the other hand it will be recalled that in the analysis shown earlier in Table III relating deafness to incidence of caloric abnormalities in subjects with vestibular symptoms there was a much higher incidence of vestibular signs than cochlear signs so that by the same token it would seem that vestibular involvement can occur in the absence of cochlear involvement.

Table VI *Relationship between perceptive and vestibular involvement*

	No. of ears	Caloric abnormalities
1 Ears without perceptive involvement	336	41/270 (15%)
2. Ears with perceptive involvement	68	16/48 (33)

The following are the main conclusions which have emerged from this analysis.

1 There is a higher incidence of women than men with otosclerosis and, furthermore, women appear to be significantly more prone than men to develop vestibular symptoms.

2. Deafness increases with age and there is greater cochlear involvement with increase in deafness, though the increase in cochlear involvement with deafness is the same both for patients with and without vestibular symptoms.

3 Patients with perceptive involvement show greater vestibular involvement.

4 Incidence of caloric abnormalities increases with deafness in patients with and without vestibular symptoms but the increase is significantly greater in the group of patients with vestibular symptoms.

5 Neither the incidence nor severity of vestibular symptoms are related to age of patient or to the degree of hearing loss.

6. The incidence of caloric abnormalities in patients with vestibular symptoms increases with their severity

DISCUSSION

The higher incidence of women than men with otosclerosis has been commented upon by other authors (Cawthorne, 1955 Shambaugh, 1956 Wolff 1958 Larsson, 1960 Hlavacek &

Chladek, 1963 Altmann et al., 1967 Soifer et al., 1970) with whose figures ours are in good agreement. There is however consider able speculation as to whether or not such figures reflect the true incidence of the disease. Guild (1944) and others (Weber 1935 Engström, 1940· Altmann et al. 1967) have shown for example that non-symptomatic otosclerotic foci are present with an equal incidence amongst men and women and on this basis it has been argued that the apparently higher incidence of *clinical* otosclerosis amongst wom en is merely a reflection of the fact that women are more likely to seek medical advice about their deafness than men. Be this as it may this explanation cannot be called in to account for the higher incidence of women than men complaining of vestibular symptoms since this incidence has occurred in patients of both sexes who *have* sought medical advice Unless one takes the view therefore that women are more likely to report vestibular symptoms than men in the course of a clinical examination it does seem that this higher incidence might well reflect an increased susceptibility amongst women.

The increase in cochlear involvement with both age and deafness might be explained by assuming that, as the disease progresses, the otosclerotic foci invade the cochlear capsule, damaging the sensori-neural element of the anterior labyrinth. There are several opinions as to how the lesion of these sensori-neural elements is brought about Rüedi (1965) and Rüedi & Spoendlin (1966) have accounted for the sensori-neural damage in otosclerosis by the existence of shunts" between the capil laries of the otosclerotic focus and the blood vessels of the adjacent area of the spiral liga ment the increased vascularity of the otosclerotic focus attracting blood via the shunts away from the capillaries of the spiral ligament thus causing atrophy of the stria vascularis and damage to the organ of Corti due to lack of adequate nutrition and oxygen. The same authors, by microchemical analysis of the perilymph from otosclerotic ears, were able to demonstrate that there was an in crease in protein and potassium content in the perilymph. This increase was due to serum permeating into the perilymph caused by venous congestion of the spiral ligament. On the other hand they were able to demonstrate a correlation between an increase in proteins and potassium with the presence of a more severe perceptive deafness. Some other authors like Schindler et al. (1965), have found an increased amount of protein and phosphatase in the perilymph of otosclerotic ears. Siebenmann (1911) Wittmaack (1919) and Altmann (1966), believe that the otosclerotic focus involving the endosteum of the cochlear capsule, could produce a toxic substance that would damage the sensori-neural elements of the cochlea.

Let us now consider the vestibular involve ment that we have been able to establish as the accompaniment of perceptive involvement. Several authors have studied the problem of how the vestibular damage originates Felisati (1958), thinks that it is due to an extension of the otosclerotic process to the vestibular labyrinthine capsule giving rise to damage to the sensori-neural elements of the otolithic maculae and cristae ampullaris. On the other hand Fisch (1965) based upon histological findings, suggests that vestibular damage is due to otosclerotic vascular or biochemical changes of the inner ear fluids.

It is interesting to point out the discrepancy between the vestibular symptoms and the vestibular signs when comparing them both with age and degree of deafness. There was an absence of correlation between the in cidence and severity of vestibular symptoms and the age of the patients as also there was none between the severity of vestibular symptoms and the degree of deafness. On the contrary as we have previously seen, there is clear relationship between vestibular signs age as well as between the degree of and the incidence of vestibular signs. ing to Wodak (1953), the despite their being related, the

vestibular symptoms follow different central pathways from those followed by the objective vestibular signs. However it is noteworthy in this context that the analysis has shown that there is a greater percentage of caloric abnormalities amongst patients with vestibular symptoms than those without.

In addition it is of some significance that, within the group of patients with subjective vestibular symptoms the majority (43%) had spontaneous paroxysmal vertiginous crises, at times similar to those observed in Menière's disease. This finding suggests that in some, an associated endolymphatic hydrops cannot be ruled out. The association of otosclerosis and Menière's disease has been suggested by several authors. Shambaugh (1959) considers that the vertiginous attacks in otosclerotic patients originates in labyrinthine involvement due to endolymphatic hydrops. He later reported in 1966 that 10% of his patients who had fenestration operations subsequently developed an endolymphatic hydrops. Furthermore Altmann & Kornfeld (1965) in a histological examination of temporal bones from patients who suffered from Menière's disease found that in some of them the dolymphatic hydrops was associated with r-cut otosclerotic lesions. Likewise Bouche al. (1968) and Martin (1968) found a typical otosclerotic stapedial ankylosis during the operation on patients with Menière's disease. Despite these findings that suggest the possibility of an association of both diseases, McCabe (1966) found otosclerotic patients with typical paroxysmal and recurrent vertiginous crises in whom he was unable to demonstrate any saccular or utricular dilatation. He described these patients as having an "otosclerotic inner-ear syndrome" clinically characterized by normal results from cochleo-vestibular and neurological examinations apart from the vertiginous attacks.

According to a number of authors (Cohen, 1959 Richards, 1964 Paparella & Chasin 1966 Piaget et al., 1969) some otosclerotic patients with paroxysmal and recurrent vertiginous crises experience a total disappearance of their vertigo after stapedectomy. Richards (1964), explains this finding by suggesting that the vertiginous crises are due to repeated rupture of the membranous labyrinth, in a labyrinth already weakened by adjacent otosclerotic foci. He believes the ruptures to result from intralabyrinthine pressure changes brought about by changes in volume of the inner ear fluids. Following stapedectomy the mobile footplate establishes a compensating mechanism that prevents the occurrence of pressure changes and therefore subsequent vertiginous attacks.

In summary therefore our findings would appear to suggest that as the otosclerotic lesions progress with the course of time, a progressive involvement of both cochlear and vestibular capsules takes place thus causing lesions of the sensory elements in the anterior and posterior labyrinths. As to the presence of vertiginous crises in otosclerotic patients it seems likely that, at least some of them, might be attributed to the extension of the otosclerotic process to the vestibular labyrinth either independent of or in association with its extension to the cochlea.

ACKNOWLEDGMENTS

The clinical material upon which this analysis is based was derived from cases referred to th National Hospital Queen Square, and examined for the most part by Dr M. R. Dix and Mr M. Spencer Harrison. Thanks are due to Mr E. Trinder for help in the initial preparation of the manuscript and to Dr J. D. Hood, Director of the Medical Research Council Hearing and Balance Unit, for his very willing advice and help throughout this study

ZUSAMMENFASSUNG

202 Patienten mit Otosklerose wurden klinisch analysiert. Mit zunehmendem Alter nahm der Hörverlust parallel zu. Der letztere war von stärkeren cochleo-vestibulären Störungen begleitet. Die Ohren mit cochlearen Störungen zeigten einen grösseren Prozentsatz kalorischer Abnormitäten als diejenigen mit reiner Leitungsschwerhörigkeit. 56 Patienten (27.7%) zeigten eine vestibuläre Symptomatologie. In dieser Gruppe

bestand keine Korrelation zwischen Alter Horverlust oder Schweregrad der Symptome. Es wird gefolgert, dass die otosklerotischen Läsionen im Verlaufe der Zeit einen progressiven Befall der cochleären sowie der vestibulären Kapseln verursachen und dass sie so Läsionen der sensori-neuralen Elemente des vorderen und hinteren Labyrinths hervorrufen. Der vestibuläre Befall kann — wenigstens in einigen dieser Patienten — das Auftreten vertiginöser Krisen erklären.

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Acta Otolaryng 73 493–501 1972

DISTRIBUTION AND CLINICAL SIGNIFICANCE OF THE AUTONOMIC NERVOUS SYSTEM IN THE HUMAN NASAL MUCOSA

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(Received July 5 1971)

Abstract. Biopsied specimens from the human inferior concha were studied histochemically to elucidate the function of the cholinergic nerve fiber adrenergic nerve fiber and myoepithelial basket cell and capillary There are rich cholinergic nerve fibers in the inferior concha. They terminate mainly in the nasal gland and the blood vessels. Lacking in adrenergic nerve fiber the nasal gland is therefore cholinergic. When the cholinergic nerve is over-stimulated by triggers, such as allergic or other reactions, the nasal gland and vessel work as a functional unit and produce profuse nasal secretion. Studies on human specimens reveal that the greater superficial petrosal nerve, vidian nerve and sphenopalatine ganglion are cholinergic in nature. The anterior ethmoid nerve also contains abundant cholinergic fibers. The nerve fibers in the vidian nerve are mainly cholinergic. The rationale for vidian neurectomy for allergic rhinitis is discussed.

Clinical and experimental observations have established that the mucous membrane of the nasal cavity is under control of the autonomic nervous system. According to Higbee (1949), vasoconstriction is a dominant effect resulting from stimulation of the sympathetic nerve fibers. On the other hand the parasympathetic nervous system is continuously making adjustments to atmospheric conditions and constitutional states in an effort to maintain the nasal membrane in a normal state. The sphenopalatine ganglion is parasympathetic and relays vasodilator and secretory fibers to the nasal mucosa

The characteristic symptoms of allergic rhinitis are rhinorrhea, nasal obstruction and sneezing, the first two of which are due to the predominant manifestation of parasympathetic nerve stimulation. Dramatic relief from the symptoms is obtained by sectioning the vidian nerve, which is the main route of the parasympathetic nerve fibers to the nasal mucosa.

This paper describes the distribution of the autonomic nerve fibers in the human inferior concha, the cholinergic fiber component in the vidian nerve and other related nerves, and the rationale for vidian neurectomy for allergic rhinitis is discussed.

The following study consists of laboratory and clinical observations.

MATERIAL AND METHODS

A piece of the inferior concha was obtained by conchotomy from 20 patients who were suffering from vasomotor rhinitis. The acetylcholinesterase, alkaline phosphatase and catecholamine were stained using histochemical techniques for the locating of the cholinergic nerve fibers, capillary and myoepithelial cells, and the adrenergic nerve fibers, respectively

The vidian nerve, the greater superficial petrosal nerve, the sphenopalatine ganglion, the anterior ethmoid nerve and maxillary nerves were obtained from cadavers stained with acetylcholinesterase.

The histochemical techniques empl described below

origin and progress. *Arch Otolaryng* (Chic.) *58* 853
— 1958. A comparison between the frequency of otosclerosis in men and women considering the age at which the first symptoms appear. *Pract Otorhinolaryng* (Basel) *20* 48

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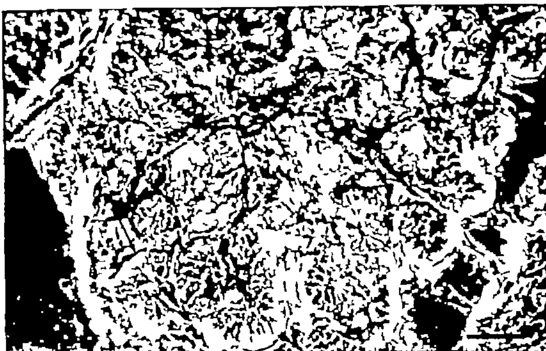


Fig. 2. Cholinergic nerve fiber (*arrows*) around the nasal glands. Human inferior concha. AChE stain. Phase contrast. 51-year-old male. Scale: 50 μ (×63).

cholinergic nerve in producing symptoms of allergic rhinitis, the patient's symptoms were evaluated before and after vidian neurectomy using a provocation test.

RESULTS

Acetylcholinesterase activity

A precipitate of brown colo[illegible]
cyankle–Hatchett's brown)
tion of acetylcholin[illegible]
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dicative of the cho[illegible]
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(Fig. 1). Control
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serous glands (Fig. 2). The precise site of its termination was not known. However there is a possibility that the nerve fiber terminates in the myoepithelial basket cell of the gland. There were no cholinergic fibers directed to or terminating in the ciliated epithelium including [illegible]goblet cells.

[illegible]nergic fibers were found in the greater
[illegible]l petrosal nerve and the vidian nerve.
[illegible] section of the vidian nerve at the
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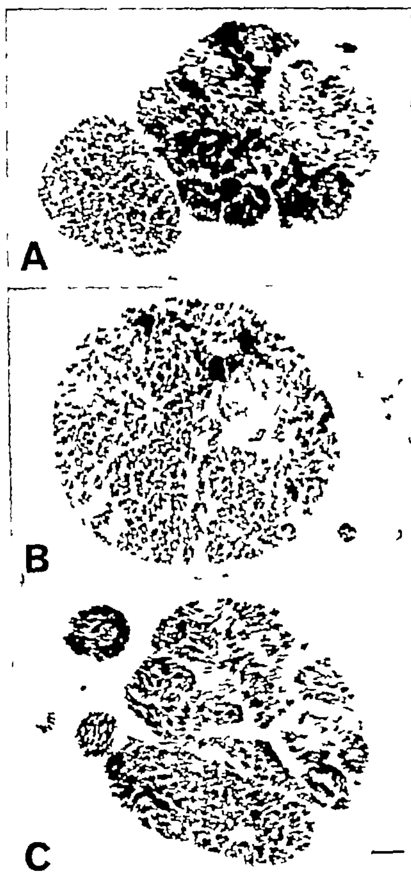


Fig 3 Cholinergic nerve fibers in the vidian nerve *A* distal portion; *B* midportion, three ganglion cells are seen; *C* proximal portion. Notice that the bundles of the vidian nerve unite and separate when passing through the vidian canal. Unstained fibers are contained. 34-year-old female. Scale: 100 μ (× 25).



Fig. 4 Ganglion cells and nerve fibers in the sphenopalatine ganglion. AChE stain. 26-year-old male Scale: 100 μ (×25).

the sphenopalatine ganglion was stained with both AChE and BChE substrate solutions (Fig. 4). Nerve fibers in the ganglion reacted negatively to BChE. The anterior ethmoid nerve contained abundant cholinergic nerve fibers, while the maxillary nerve contained less cholinergic fibers (Fig. 5)

Alkaline phosphatase activity

The reaction deposit was blue resulting from the coupling azo dye method and black from Gomori–Takamatsu's method. Control specimens were free from the reaction The capillary was made clearly visible by alkaline phosphatase staining. There are two capillary networks in the inferior concha the subepithelial capillary network and the periglandular capillary network. There was a stained structure which encircled the gland. This suggested the presence of myoepithelial basket cells (Fig. 6). However there is the possibility that the margins of the cells were stained by alkaline phosphatase.

Adrenergic nerve fiber

Adrenergic nerve fibers fluoresced when seen under a fluorescence microscope. The arteriole of the inferior concha were richly endowed with adrenergic nerves. The nasal gland was almost devoid of adrenergic nerve fibers, although fluoresced nerve fibers were occasionally seen near the gland (Fig. 7).

Case Report

A 34-year-old female complained of paroxysmal sneezing and rhinorrhea from which she had suffered for several years. The symptoms lasted for several hours daily immediately after awaking in the morning, and showed no seasonal changes. Skin testing showed a positive reaction to house dust. A diluted solution of house dust elicited profuse rhinorrhea and sneezing when applied on the mucosa of the inferior concha. Desensitization of house dust was attempted without effect. On September 25 1970, a left vidian neurectomy was performed using a transantral approach. This brought about a dramatic relief from the symptoms. The application of the house dust solution on the mucous membrane of the inferior concha on the non-operated side elicite the usual symptoms while applicatio operated side failed to bring abo



Fig 5 Abundant cholinergic fibers (*arrows*) in the anterior ethmoid nerve 51-year-old male Scale 100 μ (× 63).

and rhinorrea. Skin testing showed a positive reaction to house dust.

DISCUSSION

present study is based on the concept of ie neurohumoral theory of the autonomic nervous system. Generally speaking cholinergic fibers of the autonomic nervous system are pre and post-synaptic fibers of the parasympathetic nerve and the presynaptic fiber of the sympathetic nerve The adrenergic fiber is the postsynaptic fiber of the sympathetic nerve There are also cholinergic sympathetic fibers, such as those to the sweat glands of the skin These fibers can be identified by staining with acetylcholinesterase and catecholamine Cholinesterase may be divided into two types, specific acetylcholinesterase and non specific cholinesterase. Acetylcholinesterase is capable of hydrolysing acetylthiocholine, whereas cholinesterase hydrolyses esters of choline other than acetyl more rapidly than acetylcholinesterase. In order to demonstrate cholinesterase, it is necessary to use butyrylthiocholine instead of acetylthiocholine as a substrate (Bancroft, 1967)

The nasal glands produce mucus which protects the nasal mucosa by forming a mucous blanket. Overproduction of mucus results in nasal discharge

The nasal gland is controlled by the autonomic nervous system (Fig. 8). Secretory impulses to the nasal gland are carried by postsynaptic fibers of the sphenopalatine ganglion, where the presynaptic nerve fibers make synapses. The cell body of the presynaptic fiber is in the superior salivatory nucleus. The fiber which runs through the intermediate nerve of the facial nerve, the greater superficial petrosal nerve and the vidian nerve along with the sympathetic deep petrosal nerve finally establish synaptic contact with



Fig 6 Presence of myoepithelial basket cell. Long processes encircle two glands. Gomori–Takamatsu's alkaline phosphatase stain. 23-year-old male. Scale: 10 μ (× 400).

the perikarya of postsynaptic neurons in the sphenopalatine ganglion. We have demonstrated with human patients that the secretory nerve fibers are cholinergic. So far, the adrenergic nerve fiber of the nasal gland has not been demonstrated (Dahlström & Fuxe, 1965; Nishimura, 1970). The present study confirms this finding. Consequently, we conclude that cholinergic innervation is dominant in the functioning of the nasal gland. Nevertheless,

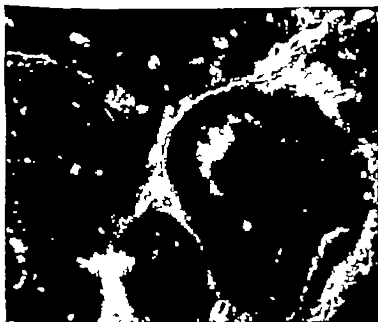


Fig 7 A fluorescent nerve fiber around the nasal gland of the human inferior concha. 20-year old female (× 160).

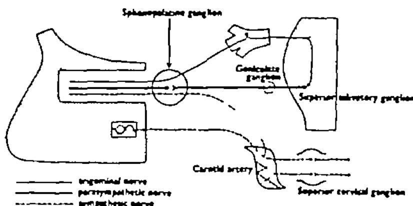


Fig. 8 Schema of the innervation of the nasal mucosa.

we cannot disregard the presence of sympathetic fibers.

The destination of the cholinergic fiber is mainly the nasal gland and blood vessels, as Ishii reported in 1970 Yet electronmicroscopic studies of the nasal gland have not ascertained the location of the cholinergic nerve fiber endings. The present study suggests the possibility that the cholinergic fiber terminates in the myoepithelial basket cell It is present in secretory units of either the mucous or the serous type The cell has a central body and many long cytoplasmic processes that encircle and grasp the secretory unit. Its cytoplasm seems contractile as in electronmicroscope studies which reveal myofibrils. Leeson (1956) by demonstrating the activity of alkaline phosphatase in rat submaxillary gland indicates that the cell is selectively stained histochemically Its function appears to squeeze the gland so as to extrude the secretion. This function is probably connected with the rich periglandular capillary network. When the cholinergic nerve is stimulated the arterioles in the concha dilate, permeability through the capillary wall changes, the uptake of serum constituents through the basement membrane into the gland increases, secretion is formed and this is finally squeezed from the gland.

The vidian nerve is said to contain both sympathetic and parasympathetic fibers. According to Fenton & Larsell (1928) and Larsell (1942) instead of one nerve there are two nerves in the vidian canal. One is the continuation of the greater superficial petrosal nerve the other is the deep petrosal nerve. The present study also demonstrates two nerve bundles at the distal extreme and two or more at the proxymal extreme of the vidian nerve. However they are stained by acetylcholinesterase, indicating that the bundles are cholinergic Furthermore, two AChE-positive bundles are found to be united into a large bundle at the midportion of the vidian nerve. By histochemistry it was demonstrated that the vidian nerve is mainly cholinergic, although a small number of fibers mixed with the vidian nerve remained unstained. There is, therefore, an interlacing of nerve fibers in the vidian canal and the canal is seen to be not merely a channel for passing nerve fibers. The separation and rearrangement of nerve fibers take place here. From the present findings, there is a possibility that the vidian nerve consists of parasympathetic cholinergic and sympathetic cholinergic fibers.

Golding Wood (1970) causally classified chronic vasomotor rhinitis into two types: atopic and non atopic (cholinergic). Histamine and acetylcholine are the chemical mediators in the atopic and cholinergic types respectively Golding Wood does not think it difficult to distinguish between these types.

From the present clinical study the role of the cholinergic nerve fiber in producing symptoms of allergic rhinitis is obvious. Since the symptoms subsided after sectioning the vidian nerve, allergic reaction in the nasal mucosa is

only a trigger which probably stimulates the trigeminal nerve and/or afferent fibers first and this stimulus is eventually relayed to the cholinergic nerve. Whether the triggers are due to allergy mechanical stimuli, thermal stimuli or whatever the symptoms are produced by the cholinergic nerve system.

Golding-Wood (1970) stated that the allergic cases are usually successfully treated by desensitization and that the cholinergic cases are candidates for vidian neurectomy only when the symptoms are severe and intractable to conservative therapy Not infrequently desensitization fails to cure allergic patients in our clinic. Irrespective of whether they are allergic or cholinergic types, vidian neurectomy is suggested for those whose symptoms are so persistent and intractable that any conservative therapy fails to abate the symptoms. This is because the cholinergic nerve plays a predominant role in both allergic and cholinergic rhinitis. The functional relation between the autonomic nervous system and IgE in the nasal mucosa must be elucidated.

ACKNOWLEDGMENT

We would like to thank Dr K. Uono of Department of Internal Medicine, University of Tokyo, for the preparation of the adrenergic nerve stain, and Dr Y Nakayama of the Children's Clinic, Kudanzaka Hospital, for running the skin tests

ZUSAMMENFASSUNG

Die Schleimhaut der unteren Nasenmuschel bei Menschen wurde im lebenden Körper histochemisch gefärbt und die Verteilung der cholinergischen Nerven, die Verteilung der myoepithelialen Korbzellen und die Verteilung der Kapillaren untersucht. In der Schleimhaut der unteren Nasenmuschel gab es reichlich cholinergische Nervenfasern, die meistens in den Nasendrüsen und in den Gefässen endeten. In den Nasendrüsen wurden keine adrenergischen, sondern cholinergische Nervenfasern beobachtet. Falls die parasympathischen Nerven durch Allergie oder durch irgend eine andere Ursache ubermässig gereizt werden, so funktionieren Nasendrüsen und -gefässe als Einheit zusammen, dann erfolgt profuse Nasensekretion. Nervus petrosus superficialis major N Vidianus und Ganglion sphenopalatinum bei Menschen sind von Natur aus cholinergisch. Nervus ethmoidalis anterior enthält auch reichlich cholinergische Nervenfasern. Die Nervenfasern des N Vidianus sind hauptsächlich cholinergisch. Ferner erörterte der Verf. das vernunftgemässe Auftreten der Neurektomie von N Vidianus für Nasenallergie.

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Acta Otolaryng 73 502–505 1972

PHOTOPALATOGRAPHY AS A DIAGNOSTIC METHOD IN PHONIATRICS

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(Received July 15 1971)

Abstract This report concerns the application of photopalatography for the diagnosis, differential diagnosis, and control of therapy in phoniatric clinical pictures. A selection of typical palatogrammes from 56 cases is discussed. Photopalatography may be re commended as a routine method in phoniatrics be cause of its small demands on time and expenditure. Furthermore it is of value for documentation and expert evidence

Hitherto palatography has been applied above all in experimental phonetics to examine tongue movements in the cavity of the mouth during speech. Palatography is gaining im portance as an additional method for the diagnosis of phoniatric diseases. In the text -oks of Arnold (1959) Seeman (1969), and -amer (1967) we find typical palatogrammes with descriptions.

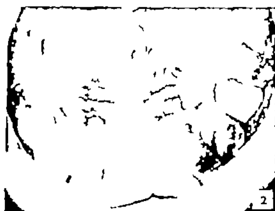
The first palatographical examinations were made by Coles (1872) in patients with defects of the palate. Scholz (1966) discussed the manifold single studies in his detailed paper on the development of palatography In principle we may distinguish two sorts of palatographic technique of investigation (*a*) direct palatography without application of an arti ficial palate and (*b*) indirect palatography which makes use of an artificial palate.

Both methods have in common that either the tongue or the palate is spread with a con trast-stain which contrasts well with the mu cous membrane—for example an X-ray con trast-medium.

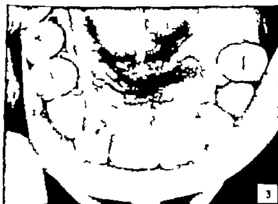
Our investigative method is direct palatography without application of an artificial palate By using the X ray contrast-medium Falibaryt (VEB Fahlberg List, Magdeburg) good palatogrammes are to be obtained after the patient s having articulated. With the use of a mirror we now view the marked contact areas in the area of the palate and eventually photograph them (Fig 1) In our investigation we restricted ourselves to the sounds *t*, *l* and *k* (Figs. 2–4). The palatographical in



Fig 1 Palatographical method of investigation.

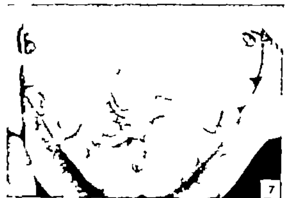












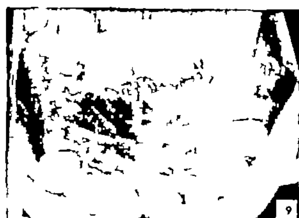
Figs. 2–4 Normal palatogrammes of the sounds *s* (*2*), *l* (*3*), and *k* (*4*).

Figs. 5–7 Palatogrammes of sigmatismus interdentalis (*5*), addentalis (*6*), and lateralis sinister (*7*).

vestigation of these sounds seemed to be sufficient for the diagnosis of the speech disturbances we examined. By the *s*-palatogramme one surveys the tongue-contact with the alveolar arches of the upper jaw the *l* palatogramme elucidates disturbances in the area of the anterior parts of the palate and the *k* palatogramme at the posterior palate area. Thus we may inspect the most essential tongue movements during articulation.

We will now describe some typical pal grammes with striking alterations of They have been chosen from a cases.







Figs. 8–10 Palatogrammes of (*8*) unilateral glossolysis (dexter *l*-sound), (*9*) extrapyramidal dysarthry (*l*-sound) and (*10*) macroglossia (*k*-sound) resulting from hypophysis adenoma.

Palatography plays an important part in the differential diagnosis of different forms of sigmatism.

In *sigmatismus interdentalis* we find a typical *s*-palatogramme The dislocation of the tongue between the slightly opened dental rows, typical of sigmatismus interdentalis, causes the settling of the contrast-medium at the cutting-edges of the incisors (Fig. 5)

In *sigmatismus addentalis* a spot of the contrast-medium can be seen at the oral side of the incisors (Fig. 6). By means of the *s*-palatogramme one can detect a contact area at the *lateral side of the median line in sigmatismus lateralis* (Fig. 7)

The next photo (Fig. 8) shows the *l*-palatogramme of a patient suffering from a *glossolysis dexter* with unilateral atrophy of the tongue The deviation from the standard is evident. Considerable changes in the palatographic picture are found in *dysarthries*, too. The *l*-sound of an adult with an extrapyramidal dysarthry and athetotic disturbance of motility (Fig 9) gives us a glimpse of the considerably altered course of articulation A dorsal dislocation of the contact areas: palate–tongue has taken place. Interesting palatographic findings are also to be seen in a female patient with extensive acromegaly and *macroglossia* resulting from a chromophobe hypophysis adenoma. The *k*-palatogramme shows us a greatly enlarged and therefore atypical contact area (*Fig 10*) *A greatly enlarged tongue* is certainly the cause of the articulatory alterations.

ZUSAMMENFASSUNG

Es wird über die Anwendung der Photopalatographie in der Diagnostik, Differentialdiagnose und Therapiekontrolle bei phoniatrischen Krankheitsbildern berichtet. Eine Auswahl typischer Palatogramme von 56 Fällen wird erörtert. Die Photopalatographie kann als Routinemethode in der Phoniatrie wegen ihres geringen apparativen und zeitlichen Aufwandes empfohlen werden. *Außerdem ist sie* fur die Dokumentation und Veranschaulichung wertvoll.

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Acta Otolaryng 73 506–512, 1972

SINOSCOPY ENDOSCOPY OF THE MAXILLARY SINUS

Technique Common and Rare Findings

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(Received July 16, 1971)

Abstract. Endoscopic examination of the maxillary sinus was introduced in 1902 but only infrequently used in the next half century partly because of the limited capacity of the optics and light source The method has been reintroduced and the technique refined in the last 20 years. The development of cold light and Hopkins optics has improved the capability of the method and now makes it possible to make excellent colour photographs for further study comparison and education. As the method gives information which is often superior to that obtained during clinical and roentgen-examination, it is essential to disseminate the knowledge of the method. The technique used and typical findings are demonstrated in *colour photographs.*

Already in 1902 Hirschmann and later the same year Reichert realized how important the recently developed Nitze cystoscope could be in the diagnosis of diseases of the maxillary sinus. They used a drilled hole in the canine fossa or a dental alveola as the preferred way of introducing the sinoscop[illegible] Spielberg (1922) simplified the method using [illegible] straight trocar through the thin median w[illegible] of the inferior meatus following local [illegible]hesia and using the same technique [illegible] for antral puncture. In spite of the ap[illegible]t advantage of the method, it was only [illegible]sed in the first half of the century (Mo[illegible]5 Lüdecke 1932 Christensen, 1946), [illegible] times by dental surgeons (Bethmann, 1[illegible] However during the last twenty years ma[illegible] authors have used the method (Bollobás, 1[illegible]4 Riccabona, 1955 Timm 1956 1965 Bauer & Wodak, 1957 1959 Hally 1960 Rosemann 1961) emphasizing the value of the endoscopy in preference to clinical and roentgen examination. In addition the method permits a biopsy to be taken from the mucosa the secretion can be removed for further examination and a polyethylene tube can be inserted for daily lavage (Schobel, 1955 Knudstrup, 1970) in cases of sinusitis. The apparent histological characteristics of the different allergic and infectious sinus diseases were studied (Bauer & Wodak, 1957 Wodak, 1958 Bauer 1960) and it was demonstrated that the mucosal changes of the sinus often differed from those occurring at the same time in the nasal mucosa (Bauer 1958).

The endoscopic pictures in different diseases were described and drawings in colour shown (Hirschmann, 1902 Bethmann 1953 Lüdecke, 1932 Bauer & Wodak, 1959 Hally 1960). In 1946 Halvor Christensen succeeded in getting colour photographs, shown at a lecture, but in 1965 Timm published the first colour photographs in a journal. The development of cold light and not least the Hopkins optics now makes it possible to get excellent photographs in colour These permit demonstration and discussion control of any change during treatment and not least may be used for educational purposes. We have therefore found it of interest to describe the technique and to demonstrate common and more rare findings in order to disseminate knowledge to this valuable method.

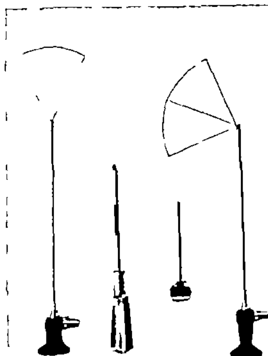


Fig. 1.

TECHNIQUE

Equipment

The Storz equipment used (Fig. 1) includes a cannula with a diameter of 4 65 mm and a trocar a Hopkins optics with a diameter of 4 mm and a field of vision of 25–115 degrees (also suitable for rhinoscopy rhinopharyngoscopy and transconioscopy) a Hopkins optics with a diameter of 2.7 mm and a field of vision of 60 degrees around the axis of the instrument, a cold light generator and fibre cable. A Zeiss Icarex reflex camera with a focal length of 90 mm and with a special fastcoupler for the optics is used for colour photography A special Storz electron flash equipment is used (Fig. 2) We find the colour film Anscocrome 500 suitable for the purpose

Anaesthesia

In most adults sinoscopy can be carried out under local anaesthesia using a 5% Lidocain solution, containing a vasoconstricting agent such as Metaoxedrine 0.1% This solution is used partly on a cotton applicator placed under the inferior turbinate partly on a piece of cotton placed in the posterior of the middle meatus under the middle turbinate This not only ensures anaesthesia but diminishes the bleeding in the sinus caused by the puncture. Premedication by means of pethidine 25 mg intramuscular is helpful in nervous adults, but general anaesthesia may be necessary especially in cases of septal deformities and abnormally thick median wall, and is always necessary in children.

Puncture

The cannula with trocar is placed lateral to the inferior turbinate and as far as possible anteriorly on the median wall of the sinus. The trocar is then rotated and at the same time pressed through the osseous wall using the same technique as that for antral puncture. When the trocar is withdrawn any secretion can be removed by means of suction and a sample taken for bacteriological examination. Repeated instillation and suction of sterile physiological saline is often useful should bleeding occur.

Sinoscopy

The endoscopy can now be introduced through the cannula, and moved to and fro and at the same time rotated, thus permitting a thorough examination of all parts of the sinus to be carried out (Fig. 3) This shows a normal sized and -shaped maxillary sinus, in which all parts except the dark spot could be seen with the 70 degree optics. The straight optics could more than compensate for this defect as shown by the white circle.

Photography

When photographs are wanted, the electron-flash bulb and the camera are attached to the endoscope (Fig. 2). The reflex camera makes it easy to observe the area to be photographed.

Biopsy

A sample of tissue can be removed for histological examination by means of fine cup biopsy forceps. As the instruments ar

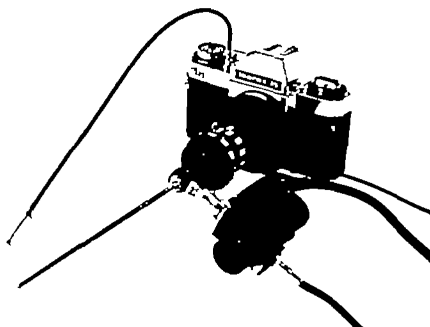


Fig. 2.

do not include movable biopsy forceps, the only area from which a biopsy can be removed is roughly that dark spot in Fig. 3 the diameter of which is determined by the angle to which the cannula can be pressed to one side or the other Efforts are now being made to solve this problem, and also to enable biopsy to be taken under direct vision.

Insertion of tube

In cases of sinusitis we use insertion of a split polyethylene tube (Fig 4) which can pass the cannula with the wings pressed together can be left in position as long as daily lavage (Fig. 5) is wanted and is invisible when not used. It can easily be removed when treatment is no longer necessary (Knudstrup, 1970) The dis-



Fig 3.

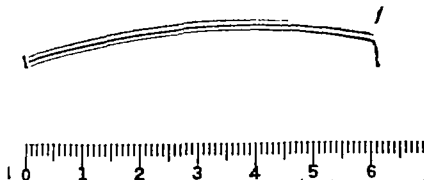


Fig 4

comfort of repeated antral punctures can be avoided with this procedure.

MATERIAL

The method has been used in our department for almost 4 years. We have been able to make colour photographs of the findings since December 1969 The experience gained in sinoscopy of 585 sinuses forms the basis for this work. The examination was successfully carried out in 510 cases (87.2%) The procedure failed or was incomplete in 75 cases (12.8%) (Table I)

Causes of failure or incomplete sinoscopy (Table I)

1 *Patient too nervous.* In some nervous adults and in all children, the procedure is so disagreeable, in spite of premedication using pethidine and carefully applied local anaesthesia, that the procedure is a failure. General anaesthesia is then necessary

2. *Anatomical reasons* It may be impossible to insert the cannula in cases of severe devia-

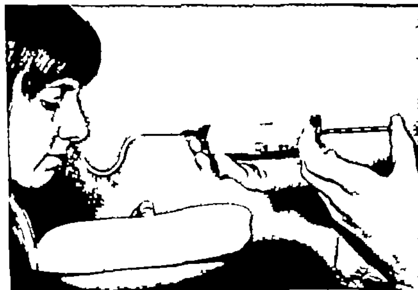


Fig 5.

Table I *Sinoscopy failing or incomplete in a series of 585 sinuses examined*

Failing, 19 cases (3.3 %)[a]			Incomplete 56 cases (9.5 %)[b]			Complication
Too disagreeable under local anaesthesia	Septum deviation	Osseous wall too hard	Sinus tissue touches lens	Bleeding spoils view	Discharge spoils view	Perf. to paraphar fossa
3	2	14	19	29	7	1

[a] Confidence limits 2.0–5.1 %.
[b] Confidence limits 7.2–12.0 %.

tion of the nasal septum or very thick osseous wall between the sinus and the inferior meatus.

3 *Sinus tissue obscuring the lens* Endoscopy cannot be successful in cases where the entire sinus is filled by tissue, but a sample of tissue and discharge can be removed for further examination and a polyethylene tube inserted in cases of sinusitis.

4 *Bleeding or discharge spoiling the view* In some cases it may be impossible to get a clear view in spite of repeated irrigation using physiological saline

SINOSCOPIC PICTURES

Normal sinus (Fig 6)

he mucosa is thin pellucid the underlying bone gives it a yellowish colour Some small vessels are seen and upwards and medially the antral hiatus. Backwards and medially is an almost circular depressed area with a blueish colour This is the thin wall between the maxillary sinus and one of the posterior ethmoidal cellulae In cases of ethmoiditis the colour can change to the yellow colour of mucopus.

Acute sinusitis (Fig 7)

The mucosa is red and swollen with dilated capillaries. Mucopus is often present

Generalized slight oedema (Fig 8)

In some cases the picture seen can be characterized by diffuse oedema and dilated greater vessels, often dark in colour The mucosal colour can be reddish. Secretion, if any is often serous. This picture is presumed to be that of an acute sinusitis during healing (Timm 1965)

Chronic sinusitis (Fig 9)

A very mixed picture can be seen in these cases with long lasting mucopurulent nasal discharge. The mucosa is thick in parts and oedematous or polypoid in other parts fibrotic. Mucopus is present.

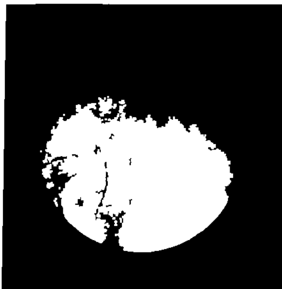
Polyposis (Fig 10)

In some cases the sinus can be more or less filled by polypoid masses. The appearance of these is often similar to that of nasal polyps and some serous secretion may be present. They are presumed to be of allergic origin. In some cases, however an infection can take place making them more reddish in colour and changing the secretion to mucopus.

The following four examples show how useful endoscopy can be in the diagnosis of a circumscribed swelling of unknown nature, all of them giving the same picture in the roentgenogrammes.

Sinusitis caused by periapical dental infection (Fig 11)

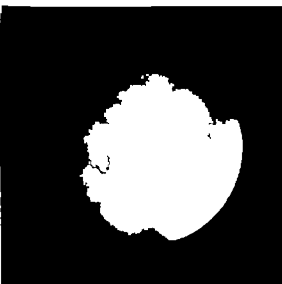
An oedematous swelling in the alveolar furrow can be seen early in the disease



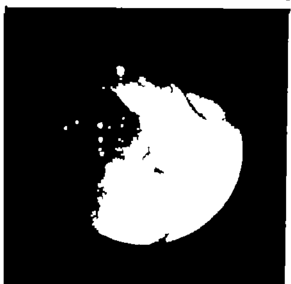
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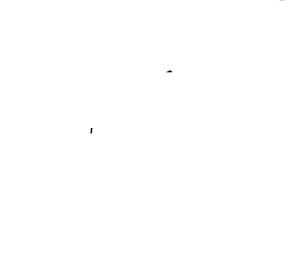
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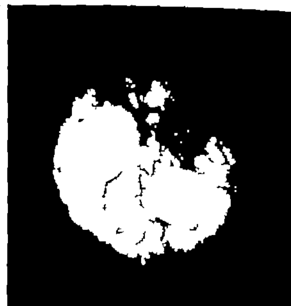
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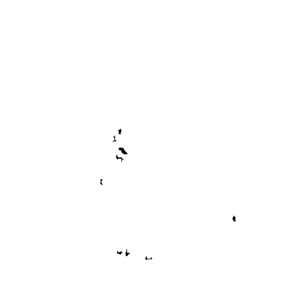


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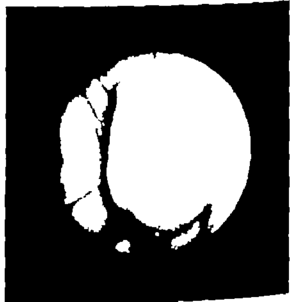












Cyst

The cyst may be thin-walled, yellowish and pellucid (Fig. 12) and in some cases reach a considerable size In such cases the wall can often be grasped by biopsy forceps and the cyst torn to pieces causing it to disappear In other cases some small white or yellow cysts are seen (Fig. 13) The wall can be covered on both sides or the outer side alone by cylindric or cubic epithelium in both types of cyst. When the inner surface is covered with epithelium the cyst appears to be caused by retention in a gland. In the first pellucid type of cyst mentioned, the content is serous fluid, in the second it may be eosinophilic material, cholesterol or pus.

Single polyp (Fig 14)

The smooth surface of grey yellowish or -reddish colour and semipellucid appearance is similar to that of nasal polyps.

Solid polyp and other non-malignant tumours (Fig 15)

This polyp shows a yellowish colour in contrast to the previous picture, is not semipellucid, and is covered with normal mucosa.

Malignant tumours

Early carcinoma of the maxillary sinus can easily be detected by sinoscopy but is rarely seen, as the tumour often gives rise to symptoms only after having spread to the nasal or oral cavity In such cases biopsy can be obtained more easily than from sinoscopy Endoscopy can, however at times give information as to the exact spread and thus be helpful in planning the treatment. This is demonstrated in Fig. 16 where in a case of rhinoparyngeal carcinoma some blurring was apparent in the posterior ethmoidal cellulae on the roentgenogrammes. Sinoscopy demonstrated spread to the cellula.

Sinoscopy can also be helpful in controlling the effect of a treatment, including early detection of a relapse. The biopsy technique is, as earlier mentioned, of limited use in its present state.

Ectopic tooth (Fig 17)

In this case an ectopic tooth was found situated close to the sinus in the roentgenogrammes. The exact position of the tooth was ascertained as transantral removal was planned.

CONCLUSION

Endoscopy of the maxillary sinus can be helpful as it gives more exact information on the condition of the mucosa and the adjacent structures, thus permitting the correct method of treatment to be chosen with greater certainty than when relying on examination of the nasal cavity X-rays and antral puncture alone. The method permits colour photography and is thus helpful with demonstrations, discussions and for education. Sinoscopy is almost as easy to carry out under local anaesthesia as the usual antral puncture and does not entail much more inconvenience for the patient. The present equipment is not completely satisfactory for biopsy Studies comparing the results obtained during sinoscopy and those obtained during roentgen and histological examination will be published later

ACKNOWLEDGMENT

Rask-Ørsted Foundation, Simonsen & Weel, Copenhagen, and Storz, Tuttlingen, have given financial support to the publication of the colour photographs etc., for which we wish to express our gratitude.

ZUSAMMENFASSUNG

Die endoskopische Untersuchung der Kieferhöhlen wurde 1902 eingeführt, wurde aber in den folgenden 50 Jahren nur selten verwendet, hauptsächlich wegen unzulänglicher Optiken und Lichtquellen. Die Methode ist in den letzten 20 Jahren wiedereingeführt und verfeinert worden. Die Entwicklung kalten Lichtes und die Fiberoptiken von Hopkins haben jetzt die Methode leicht verwendbar gemacht und es ermöglicht glänzende Farbfotografien der Schleimhaut für weitere Studien, Vergleiche und [illegible] Unterricht herzustellen. Die Methode
die oft besser sind, als die, die

Untersuchung und Röntgenbilder der Nebenhöhlen erreicht werden, es ist deshalb wichtig, die Kenntnis der Methode zu verbreiten. Die verwendete Technik und die typischen Funde sind durch Farbenbilder demonstriert worden.

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Acta Otolaryng 73 513–520 1972

EMBRYONAL RHABDOMYOSARCOMA IN THE EXTERNAL EAR

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(Received July 25 1971)

Abstract The authors describe two cases of embryonal rhabdomyosarcoma in the external ear: one in a girl, 9 years of age, with a rapid lethal clinical course in spite of repeated wide surgical excisions, and the other in a female, 26 years of age, radically treated for two recurrences and still alive 5 years after the appearance of the tumour. The differential diagnosis in the ear region between embryonal rhabdomyosarcomas and fetal rhabdomyomas, a new tumour entity with a predilection for the ear (Enzinger), is especially considered. The therapy and factors influencing the prognosis are discussed.

Rhabdomyomatous tumours are of special interest in several respect to otolaryngologists. In accordance with the nomenclature recommended by the World Health Organization (Enzinger et al., 1969) rhabdomyosarcomas can be divided into the following three groups: pleomorphous, alveolar and embryonal types. Among the embryonal rhabdomyosarcomas, sarcoma botryoides is a subgroup with respect to gross (polypous, coarsely lobulated) and microscopical appearances. The principal primary sites of embryonal rhabdomyosarcomas include the urogenital tract and certain parts of the head, especially the nasopharynx and the orbit (cf Porterfield & Zimmermann, 1962 Dito & Batsakis, 1962, Masson & Soule, 1965 Stout & Lattes, 1967) It has been proposed that the embryonal rhabdomyosarcoma is the most common malignant tumour in the head and-neck region in children (Albores-Saavedra et al., 1965). Stout & Lattes (1967) combine botryoid sarcoma, embryonal rhabdomyosarcoma, and alveolar rhabdomyosarcoma in the term juvenile rhabdomyosarcoma. The concept of alveolar rhabdomyosarcoma as a distinct entity is, however fortified by its characteristic location and the age incidence: the alveolar type is predominantly found in the musculature of the extremities and mainly affects adolescents and young adults It is almost always intimately associated with striated musculature (Enzinger & Shiraki, 1969).

Embryonal rhabdomyosarcoma is often located in the auditory canal (cf Stout & Lattes, 1967) and may occur in the middle or internal ear and mastoid bone (Dito & Batsakis, 1962 Myeras et al. 1968 Gross et al., 1969: Haglund et al., 1970: Jaffe et al., 1971). However judging from the literature, this tumour seems to be exceedingly rare in the external ear

The present paper describes two cases of embryonal rhabdomyosarcoma located in the external ear: one tumour was relatively poorly differentiated and seen in a young girl, the other was moderately well differentiated and observed in a young adult female.

CASE REPORTS

Case 1

In January 1968, a 9-year-old girl presented with a slowly growing tumour of the right ear lobe of 3 months duration. The tumour was excised and diagnosed as a richly cellular, malignant, mesenchymal tumour. As

of this diagnosis the patient was taken to an ENT ward where a wider excision was made including part of the external ear the chondromatous part of the outer ear-canal, and lymph nodes behind the angle of the mandible. The specimen revealed a rather extensive tumour that was to all appearances radically excised with no sign of lymph node metastasis. Four months later the malignancy recurred and an attempt at radical surgery was made at the ENT Department, Sahlgren s Hospital Gothenburg. The excision included the rest of the external ear the surrounding skin the parotid gland, and the tissues of the neck on the same side. Histopathological examination revealed extensive tumour growth with infiltration of the parotid gland and surrounding lymph nodes. It was difficult to judge whether the tumour had been completely excised. After another 4 months further recurrence was observed, but radical excision was no longer possible. After this, the tumour grew to an enormous size and the patient died about 16 months after the appearance of the initial symptoms. At post mortem examination there was at the *right side of the neck* a tumour the size of a child s head with infiltration towards the base of the skull, the pharynx larynx with growth into the trachea. stases were found in the lungs.

ase II

A 26-year-old woman in November 1965 discovered a swelling on her right ear lobe The rate of growth was fast. In 3 weeks the tumour reached the size of a finger-tip. The tumour was excised but recurred 4 months later A new excision was made and preliminary histological examination revealed a malignant *mesenchymal tumour* Because of this diagnosis a wider surgical excision was made at the ENT Department, Sahlgren s Hospital, Gothenburg, including the soft tissue surrounding the site of the primary tumour Microscopic examination showed no residual tumour in this specimen. Two years after the first symptoms, a lymph node at the anterior margin of the right trapezius muscle and a mass measuring $2.2 \times 1.0 \times 0.9$ cm in the area of the earlier operations were removed. The histopathological examination showed recurrence of the tumour but the lymph node was free from metastasis. At this stage the external ear along with the tissues behind and beneath the ear including the upper part of the sterno-cleido-mastoid muscle and part of the parotid gland was removed. Resection of the facial nerve was made and reconstructed by an end to end suture of the remaining parts. Mastoid process was cut so deeply that only the inner cortical bone was left. Microscopic examination revealed no tumour growth. Six months later a few enlarged lymph nodes on both sides of the neck were found and radical neck dissection on the right side was performed. There were no histological signs of malignancy in the excised tissue. So far the patient has been well with good function in the right facial nerve and has no sign of recurrence 5 years after the onset of the disease.

PATHOLOGY

Gross appearance

In Case I the initial tumour was approximately $1 \times 1\,^1/_2$ cm in size and showed merely the appearance of an ill-defined, slightly elevated tumour in the soft tissues of the external ear The final recurrence appeared as a huge, lobulated, polypoid mass (Figs. 1 2) with a pinkish-grey to white fleshy cut-surface, of rubbery to soft myxomatous consistency with areas of cystic degeneration and yellowish and hemorrhagic necrosis. The tumour in Case II appeared at first as a slight swelling of the ear lobe measuring $1 \times 1 \times {}^1/_2$ cm. The cut surface of this tumour and the *following* two local recurrences showed ill-defined nondescript greyish-white tumour tissue.

Histological methods

The operative specimens were fixed in 10% formaldehyde solution and embedded in paraffin. Five μ thick sections were routinely



Fig. 1. Case I. The tumour on the right side of the neck 2 / months after the appearance of the final recurrence.



Fig. 2. Case I. The tumour 1 / months later than in Fig. 1 and 1 month before the death of the patient.

stained according to the haematoxylin–van Gieson method and with haematoxylin and eosin. Laidlaw's silver impregnation was used for the demonstration of reticulin fibres, and Weigert's elastin method for studying elastic tissue of tumour vessels. The periodic acid Schiff reaction (McManus) was performed with and without prior treatment of the sections with diastase (Merck). The tumour cells were examined for cross-striation as seen in rhabdomyoblasts with Masson's trichrome stain and Heidenhain's iron-haematoxylin stain.

Microscopic findings

The cellular composition of the tumour in Case I varied considerably: areas of great cellularity alternated with areas with a less cellular and more mucoid appearance. The tumour cells grew in an ill-defined nodular pattern with the covering epidermis showing necrosis and ulceration, and in other areas, irregular acanthosis and hyperkeratosis (Fig. 3). Areas composed of immature round or spindle-shaped cells with scanty cytoplasm (Fig. 4) alternated with loosely textured oedematous areas. Intermingled with these immature cells were a varying number of typical strap- and ribbon-shaped rhabdomyoblasts with considerable amounts of elongated well-defined cytoplasm showing distinct longitudinal lamination or striation. The nuclei of these cells showed elongated narrow or pointed ends and were rich in chromatin (Fig. 5). Individual rhabdomyoblasts showed definite cross-striation (Figs. 5, 6, arrows). In addition to these cells were round and large, typical racquet or tadpole-shaped, sometimes multi





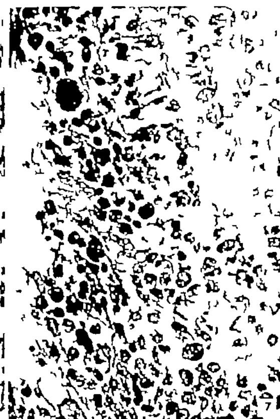




Fig 3 Case I The surface of the tumour in Figs. 1 and 2. The tumour cells grow in an ill-defined nodular pattern with the covering epidermis showing irregular acanthosis and hyperkeratosis (van Gieson, × 30).

Fig 4 Case I Tumour-area composed of immature round- or spindle-shaped cells with scanty ill-defined cytoplasm (van Gieson, × 190).

Figs. 5 and 6 Case I. Strap- and ribbon-shaped rhabdomyoblasts with their cytoplasm showing distinct longitudinal lamination or striation and cross-striation (*arrows*) (Masson trichrome stain, × 190 resp. × 300).



7 *Case II* "Round rhabdomyoblasts" of varying with well-demarcated cytoplasm showing in some distinct concentric lamination (Haematoxylin-eosin, × 190).



Fig. 8. Case II Immature, round, ovoid or spindle-shaped cells with ill-defined often vacuolated cytoplasm (Haematoxylin-eosin, 190).

nt rhabdomyoblasts with one or more centrically placed atypical nuclei. The mi c frequency varied but was moderate throughout the whole tumou The tumour showed pronounced lymphoge pread with wide-open lymph-capillaries filled ith tumour cells. The metastatic lesions in lymph nodes and lungs preser d essentially the same histologic cell picture a the primary tumour but typical strap- or ribbon-shaped rhabdomyoblasts were more freq ntly seen.

In Case II the mi scopic examination of the initial tumour and the recurrences revealed tumour cells tending to form an ill-defined nodular growth pattern. The tumour was com posed predominantly of typical, well-differentiated, small and large round rhabdomyoblasts (Fig. 7) with ample well-demarcated cytoplasm showing distinct concentric lamination and one or more nuclei which were marginally placed, often irregularly shaped, hyperchromatic, and in some cells probably pyknotic. Distributed among these tumour cells were a varying number of slightly more immature, round, ovoid or spindle-shaped cells with a scanty ill-defined, often vacuolated cytoplasm (Fig. 8) The nuclei of these cells were rather large, distinctly and often irregularly outlined containing a moderate amount of finely dispersed chromatin. Nucleoli when visible were inconspicuous. The mitotic frequency throughout the whole tumour was rather low with occasional mitotic figures. The cytoplasm of the well differentiated, round, ribbon-shaped rhabdomyoblasts showed a pronounced eosinophilia with

eosin stain picrinophilia with the van Gieson stain and a deep red colour with Masson trichrome stain. Small quantities of intracellular diastase-sensitive PAS-positive material, probably glycogen were demonstrated. The supporting stroma in both cases was composed of a varying amount of collagen-fibrous trabeculae, which stained bright blue with Masson trichrome stain. The special staining for reticulum showed a large amount of fine reticular fibres surrounding single or small groups of cells. The vascular beds were composed of primitive capillary-like vessels in both cases, and in Case II there were also some vessels showing smooth muscle cells in their walls.

COMMENTS

The clinical symptoms in patients with embryonal rhabdomyosarcoma are usually uncharacteristic and often only a rather fast growing mass is observed (Dito & Batsakis, 1962 Lawrence et al., 1964 Soule et al., 1969). The tumour is often rather well demarcated and gives no suspicion of malignancy clinically as in our two cases, where the pre-operative diagnoses were fibroma and ... cyst respectively The morphologic ...rance of embryonal rhabdomyosarcoma ... been described previously in detail (Stobbe & Dargeon, 1950 Horn & Enterline, 1958 Lawrence et al., 1964) Our two cases seem to agree very well with these descriptions. However when comparing these two cases with each other there are some interesting differences. The tumour in Case I was poorly differentiated and composed chiefly of undifferentiated immature cells. Intermixed with these cells were characteristic strap- and ribbon-shaped rhabdomyoblasts, a few of them with definite cross-striation leaving no doubt about the classification of this tumour These kinds of rhabdomyoblasts were more frequently seen in the metastatic lesions, thus indicating a higher differentiation, a phenomenon which is inconsistent with the findings of other authors (Albores-Saavedra et al., 1965) The tumour in Case II was more highly differentiated and was composed largely of round, laminated cells, which have been classified as "round rhabdomyoblasts" (Pack & Eberhard, 1952, Horn & Enterline, 1958 Lawrence et al. 1964 Enzinger & Shiraki, 1969).

On a histologic basis, embryonal rhabdomyosarcoma has been confused with many other tumours, malignant as well as benign. Moore & Grossi (1959) gave a list of 12 different initial diagnoses in their series. Some investigators studying embryonal rhabdomyosarcomas in the head region emphasize their resemblance to malignant neurogenic tumours (Porterfield & Zimmermann 1962, Haglund et al., 1970) Another apparently new tumour entity[1] is the fetal rhabdomyoma which in most cases has been located in the external ear (Enzinger personal communication) All the tumours in Enzinger's series showed a high differentiation. Thus, striated muscle fibres were easily demonstrated and mitosis exceedingly rare Enzinger points out the importance of distinguishing between fetal rhabdomyoma which has a benign clinical course, and the highly malignant, in most cases rapidly lethal, embryonal rhabdomyosarcoma. We question whether fetal rhabdomyomas have been included in some series of embryonal rhabdomyosarcomas, cf the cases of the so-called differentiated embryonal rhabdomyosarcomas in Porterfield & Zimmermann's (1962) series and cases 31 and 32 in Kauffman & Stout's (1965) series of congenital rhabdomyosarcomas.

The prominent tendency for embryonal rhabdomyosarcomas to recur is well-documented (Stout, 1946 Dito & Batsakis, 1962, Lawrence et al. 1964 Martin et al., 1965 Soule et al. 1969) In our cases there were repeated local recurrences in spite of rather extensive surgery and in Case II a second recurrence appeared after a free interval of

[1] A paper on this new tumour entity is forthcoming (Dehner & Enzinger).

almost 2 years. The clinical course in Case I was dominated by the final local recurrence on the right side of the neck, which grew to enormous dimensions before signs of more wide-spread tumour extension appeared.

It is generally agreed that aggressive surgical ablation of embryonal rhabdomyosarcoma would appear to be the treatment of choice (Dito & Batsaki, 1962 Masson & Soule, 1965 Soule et al., 1969) The metastatic spread of rhabdomyosarcoma takes place via the blood stream particularly to the lungs and bones. Embryonal rhabdomyosarcomas are also capable of metastasizing to lymph nodes (Horn & Enterline 1958 Martin et al., 1965 Masson & Soule, 1965 Davis et al., 1969) and several investigators point out the importance of lymph node dissection, if possible *en-bloc*, at the *time of initial surgical treat* ment (Horn & Enterline, 1958 Dito & Batsaki, 1962, Masson & Soule 1965 Martin et al., 1965) However others are more hesitant about the benefit of this procedure (Stobbe & Dargeon, 1950), and state that the location of the tumour and the clinical findings in the lymphatic bed must affect the choice of operation (Lawrence et al., 1964) Radical lymph node dissection was done only in Case II, but not until 6 months after the last recurrence, when enlarged, clinically suspicious lymph nodes appeared on the neck

Different opinions are expressed in the literature concerning irradiation and/or chemotherapy Some authors frankly state that most or all embryonal rhabdomyosarcomas are radiosensitive while others find only palliative effects or no results at all with irradiation (Stout, 1946 Horn & Enterline 1958 Moore & Grossi, 1959 Pinkel & Pickren, 1961 Porterfield & Zimmermann, 1962 Masson & Soule, 1965 Albores-Saavedra et al., 1965). Lawrence et al. (1964) listed 12 different chemical agents used in patients who received chemotherapy and recently Sutow (1969) has explored the potentialities of chemotherapy in the therapeutic attack. In none of our cases

evident that further experience is necessa[ry in] order to evaluate the effect of such therap[y on] embryonal rhabdomyosarcomas.

The prognosis for juvenile rhabdomy[osar]coma is poor with a 5 year survival rate [be]tween 5–15% (Dito & Batsakis, 1962 [Law]rence et al., 1964 Martin et al., 1965 S[oule] et al., 1969) The poorest survival rate oc[curs] in children with alveolar rhabdomyosarc[oma] and the best among children with sarc[oma] botryoides, and an intermediate survival among children with embryonal rhabdom[yo]sarcoma (Sutow et al., 1970) Embry[onal] rhabdomyosarcoma affecting limbs and [limb] girdles have better prognosis than embry[onal] rhabdomyosarcoma in the head-and-neck [re]gion which, except for those located to [the] orbits, have the poorest prognosis. Chil[dren] younger than 7 years of age have a be[tter] prognosis than older ones (Dito & Bats[akis], 1962, Lawrence et al., 1964 Sutow et [al.,] 1970) Another factor of prognostic sig[nifi]cance is the clinical stage of the disease at [the] time of initial diagnosis and treatment (Su[tow] et al., 1970).

Thus, wide surgical excision should be [per]formed for the control of such an aggres[sive] rapidly growing and metastasizing neoplasm [as] the embryonal rhabdomyosarcoma. A tr[eat]ment of this kind, particularly in children [and] when the tumour is located in the head, is [dis]figuring but this must be accepted in trea[ting] a lesion as malignant as embryonal rhab[do]myosarcoma. The treatment, however sho[uld] be correlated with the factors which may [af]fect the prognosis, such as the age of the [pa]tient, differentiation mitotic activity and [the] location of the tumour and the clinical st[age] of the disease.

ACKNOWLEDGMENT

We are deeply indebted to Dr Franz Enzinger [,] chief of the Soft Tissue Branch, the Armed Fo[rces] Institute of Pathology Washington, D.C., [for his] kindness letting one of us (Angervall) [...] his investigation on fetal rhabdomyoma

ZUSAMMENFASSUNG

Die Verfasser beschreiben zwei Fälle von embryonalen Rhabdomyosarcoma des äusseren Ohres. Der erste Fall ist ein Mädchen, neun Jahre alt, mit schnellem, lethalem Krankenverlauf obwohl man mehrere grosse Excisionen gemacht hat. Der zweite Fall ist eine junge *Frau, 76 Jahre alt, die nach zwei Rezidiven radikal* operiert wurde und noch fünf Jahre nach der Entdeckung des Tumors ohne Zeichen von Metastasen am Leben ist. Die Differentialdiagnosen in der Region des äusseren Ohres zwischen embryonalen Rhabdomyosarcom und fetalen Rhabdomyom einer neuen Tumorgruppe mit Hauptlokalisation auf dem äusseren Ohr (Enzinger), wird speziell beachtet. Die Therapie und Faktoren, die die Prognose beeinflussen, werden diskutiert

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